



TOXICOLOGY OF THE GASTROINTESTINAL TRACT

SECOND EDITION

Edited by
Shayne C. Gad



CRC Press
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Second Edition



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Preface

Toxicology of the Gastrointestinal Tract focuses on the specifics of the toxicology of the gastrointestinal tract—of adverse effects of xenobiotic agents and pharmaceuticals on its structure and function. Starting with an overview of basic aspects of structure and function, the book's chapters proceed to taking focused looks on specific issues. The book is also concerned with a critical appraisal of what we have come to know about the interaction of chemicals and drugs with this critical organ system and of the experimental methods and attempts to identify those regions of research that should prove to be productive for our future efforts. Within these two broad objectives, the volume focuses on a number of specific areas of intestinal research. These areas of research reflect the increasing awareness and documentation of the multiple roles of the GI tract's multiple components, starting with its well-recognized and major roles of nutrient absorption and as a protective barrier. In addition, emphasis is given to the expanding body of knowledge that relates to the intestines as a major metabolic and immunologic organ involved in the synthesis and degradation of both natural and foreign substances. A further dimension is the inclusion of focused overviews of the function and dysfunction of the human absorptive processes and the effects of microbial flora on these processes, and of specific classes of toxicants that target the GI tract.



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CHAPTER 1

Introduction
*The Gastrointestinal (GI) Tract as a Barrier
and as an Absorptive and Metabolic Organ*

Shayne C. Gad

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The GI tract is the greatest of the three major pathways into and out of the body, the other two being the skin and the respiratory tract. It is the great highway through the body, but much more. Like the other two, it is also a major metabolic organ and a major interface of the environment with the immune/lymphatic system.

This work, as a whole, focuses on the specifics of the toxicology of xenobiotics on the GI tract and adverse effects on its structure and function. It is also concerned with a critical appraisal of what we have come to know about the interaction of xenobiotics with this organ system and of the experimental methods and attempts to define those aspects of research that have proven to be productive for further understanding. Within these two broad objectives, the volume focuses on a number of specific aspects of intestinal toxicity. These areas of research reflect the increasing awareness and documentation of the multiple roles of the GI tract’s multiple components, including the well-recognized and major roles of nutrient absorption, of barrier penetration, and of the importance of the intestinal microbiome (the community of commensal microorganisms that inhabit the GI tract). In addition, emphasis is given to the expanding body of knowledge that relates to the intestines as a major metabolic and immunologic organ involved in the synthesis and degradation of both natural and foreign substances. A further dimension is the inclusion of several discussions aimed directly at the function and dysfunction of the human absorptive processes and the effects of microbial flora on these processes.

The differences between model species are also examined in depth in a separate chapter.

A wide range of approaches and methodologies have been employed to evaluate the maturation and functions of the gastrointestinal tract. Because of its unusual morphology, the mouth, intestines, and stomach can be examined *in vivo* and *in vitro* by a variety of techniques, each providing different kinds of relevant information and understanding. The expanded availability of both imaging technologies, sensors, and miniature cameras that can transit the GI tract and collect data and images at selected points has vastly expanded our ability to examine the interior and function of the GI tract at will, making potential *in vivo* evaluation much more extensive. Utilization of sacs, loops, rings, and cells have provided ample data to reveal the importance of the gastrointestinal tract as a barrier and absorptive organ and also as a highly active major metabolic tissue. Examination of the development of intestinal enzymes and metabolic pathways reveals an understanding of nutrient active transport and metabolism as well as metabolism of foreign substances. The relatively large mass of this organ system provides additional significance to its metabolic roles in the homeostasis of the organism as a whole.

Since ingestion is a major route of exposure to foreign substances, continued research involving the absorption and metabolism of these substances is needed. Currently, more Americans, Europeans, and Chinese are affected by serious diseases of the gastrointestinal tract than any other system except the cardiovascular system, which provides emphasis for the increasing concern for understanding the possible environmental contributions to gastrointestinal disease.

STRUCTURE

The gastrointestinal (GI) tract, or alimentary canal (shown in [Figure 1.1](#)), is a continuous tube that extends from the mouth to the anus through the ventral body cavity. Organs of the gastrointestinal tract include the mouth, most of the pharynx, esophagus, stomach, small intestine, and large intestine. The length of the GI tract taken from an adult cadaver is about 9 m (30 ft). In a living person, it is much shorter because the muscles along the walls of GI tract organs are in a state of tonus (sustained contraction) (Guyton and Hall, 2011; Kumer et al., 2014; Sodeman and Sodeman, 1974; Tortura and Grabowski, 2003). It should be noted that the associated digestive organs are the teeth, tongue, salivary glands, liver, gallbladder, and pancreas.

The GI tract contains and processes food from the time it is eaten until it is digested and absorbed or eliminated. In portions of the GI tract, muscular contractions in the wall physically break down the food by repetitive mixing. The contractions for mixing also help to dissolve foods by mixing them with fluids secreted into the tract. Enzymes secreted by accessory structures and cells that line the tract break down the food chemically. Wavelike contractions of the smooth muscle in the wall of the GI tract propel the food along the tract, from the esophagus to the anus.

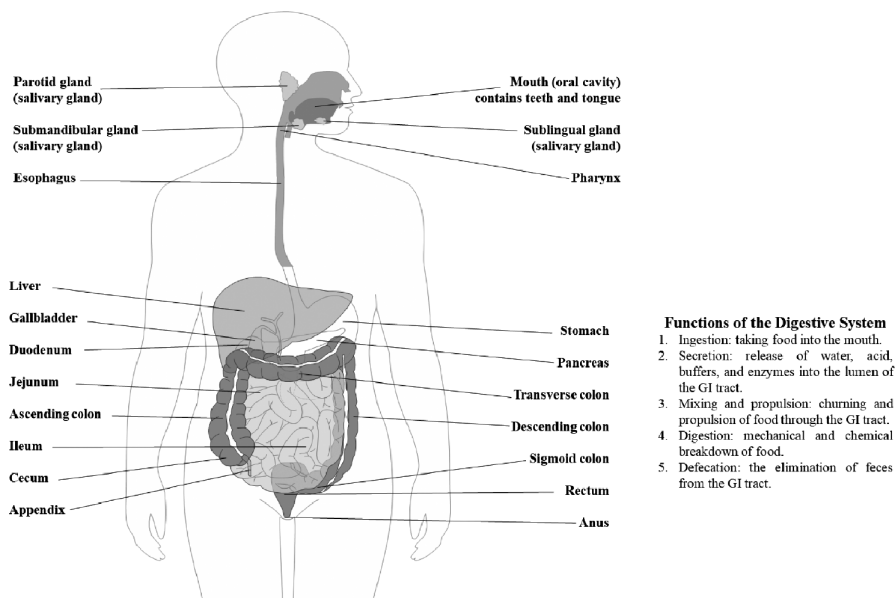


Figure 1.1 Right lateral view of head and neck and anterior view of trunk.

Overall, the digestive system performs six basic processes:

1. **Ingestion.** This process involves taking foods and liquids into the mouth (eating).
2. **Secretion.** Each day, cells within the walls of the tract and accessory digestive organs secrete a total of about 7 L of water, acid, buffers, and enzymes into the lumen of the tract.
3. **Mixing and propulsion.** Alternating contraction and relaxation of smooth muscle in the walls of the GI tract mix food and secretions and propel them toward the anus. This capability of the GI tract to mix and move material along its length is termed motility.
4. **Digestion.** Mechanical and chemical processes break down ingested food into small molecules. In mechanical digestion the teeth cut and grind food before it is swallowed, and then smooth muscles of the stomach and small intestine churn the food. As a result, food molecules become dissolved and thoroughly mixed with digestive enzymes. In chemical digestion the large carbohydrate, lipid, protein, and nucleic acid molecules in food are split into smaller molecules by hydrolysis. Digestive enzymes produced by the salivary glands, tongue, stomach, pancreas, and small intestine catalyze these catabolic reactions. A few substances in food can be absorbed without chemical digestion. These include amino acids, cholesterol, glucose, vitamins, minerals, and water.
5. **Absorption.** The entrance of ingested and secreted fluid, ions, and the small molecules that are products of digestion into the epithelial cells lining the lumen of the GI tract is called absorption. The absorbed substances pass into blood or lymph and circulate to cells throughout the body.

6. **Defecation.** Wastes, indigestible substances, bacteria, cells sloughed from the lining of the GI tract, and digested material that were not absorbed leave the body through the anus in a process called defecation. The eliminated material is termed feces.

Organs of the gastrointestinal (GI) tract are the mouth, pharynx, esophagus, stomach, small intestine, and large intestine. Accessory digestive organs are the teeth, tongue, salivary glands, liver, gallbladder, and pancreas.

The wall of the GI tract from the lower esophagus to the anal canal has the same basic, four-layered arrangement of tissues. The four layers of the tract, from deep to superficial, are the mucosa, submucosa, muscularis, and serosa.

Mucosa

The mucosa, or inner lining of the tract, is a mucous membrane (as are the other surfaces of externally communicating body channels or orifices). It is composed of a layer of epithelium in direct contact with the contents of the tract, connective tissue, and a thin layer of smooth muscle (Russ and Pawlina, 2015).

The epithelium in the mouth, pharynx, esophagus, and anal canal is mainly non-keratinized stratified squamous epithelium that serves a protective function. Simple columnar epithelium, which functions in secretion and absorption, lines the stomach and intestines. Neighboring simple columnar epithelial cells are firmly sealed to each other by tight junctions that restrict leakage between the cells. The rate of renewal of GI tract epithelial cells is rapid (5 to 7 days.) Located among the absorptive epithelial cells are exocrine cells that secrete mucus and fluid into the lumen of the tract, and several types of endocrine cells, collectively called enteroendocrine cells, that secrete hormones into the bloodstream.

The *lamina propria* is areolar connective tissue containing many blood and lymphatic vessels, which are the routes by which nutrients absorbed into the tract reach the other tissues of the body. This layer supports the epithelium and binds it to the *muscularis mucosae*. The *lamina propria* also contains the majority of the cells of the mucosa-associated lymphatic tissue (MALT). These prominent lymphatic nodules contain immune system cells that protect against disease. MALT is present all along the GI tract, especially in the tonsils, small intestine appendix, and large intestine, and it contains about as many immune cells as are present in all the rest of the body. The lymphocytes and macrophages in MALT mount immune responses against microbes, such as bacteria, that may penetrate the epithelium. It should be noted that current ICH and FDA guidance suggests examination of the MALT tissues when evaluating potential immunotoxicity (Gad, 2015).

A thin layer of smooth muscle fibers called the *muscularis mucosae* throws the mucous membrane of the stomach and small intestine into many small folds, which increase the surface area for digestion and absorption. Movements of the *muscularis mucosae* ensure that all absorptive cells are fully exposed to the contents of the gastrointestinal tract.

Submucosa

The submucosa consists of areolar connective tissue that binds the mucosa to the muscularis. It contains many blood and lymphatic vessels that receive absorbed food molecules. Also located in the submucosa is the submucosal plexus or plexus of Meissner, an extensive network of neurons. The neurons are part of the enteric nervous system (ENS), the “brain of the gut.” The ENS consists of about 100 million neurons in two plexuses that extend the entire length of the tract. The submucosal plexus contains sensory and motor enteric neurons, plus parasympathetic and sympathetic postganglionic neurons that innervate the mucosa and submucosa. Enteric nerves in the submucosa regulate movements of the mucosa and vasoconstriction of blood vessels. Because its neurons also innervate secretory cells of mucosal and submucosal glands, the ENS is important in controlling secretions by the GI tract. The submucosa may also contain glands and lymphatic tissue.

Muscularis

The muscularis of the mouth, pharynx, and superior and middle parts of the esophagus contains skeletal muscle that produces voluntary swallowing. Skeletal muscle also forms the external anal sphincter, which permits voluntary control of defecation. Throughout the rest of the tract, the muscularis consists of smooth muscle that is generally found in two sheets: an inner sheet of circular fibers and an outer sheet of longitudinal fibers. Involuntary contractions of the smooth muscle help break down food physically, mix it with digestive secretions, and propel it along the tract. Between the layers of the muscularis is a second plexus of the enteric nervous system—the myenteric plexus or plexus of Auerbach. The myenteric plexus contains enteric neurons, parasympathetic ganglia, parasympathetic postganglionic neurons, and sympathetic postganglionic neurons that innervate the muscularis. This plexus mostly controls tract motility, in particular the frequency and strength of contraction of the muscularis.

Serosa

The serosa is the superficial layer of those portions of the GI tract that are suspended in the abdominopelvic cavity. It is a serous membrane composed of areolar connective tissue and simple squamous epithelium. As we will see shortly, the esophagus, which passes through the mediastinum, has a superficial layer called the adventitia composed of areolar connective tissue. Inferior to the diaphragm, the serosa is also called the visceral peritoneum; it forms a portion of the peritoneum, which we examine in detail next.

The peritoneum is the largest serous membrane of the body; it consists of a layer of simple squamous epithelium (mesothelium) with an underlying supporting layer of connective tissue. The peritoneum is divided into the parietal peritoneum, which lines the wall of the abdominopelvic cavity, and the visceral peritoneum, which covers some of the organs in the cavity and is their serosa. The slim space between

the parietal and visceral portions of the peritoneum is called the peritoneal cavity, which contains serous fluid. In certain diseases, the peritoneal cavity may become distended by the accumulation of several liters of fluid, a condition called ascites. As we will see, some organs lie on the posterior abdominal wall and are covered by peritoneum only on their anterior surfaces. Such organs, including the kidneys and pancreas, are said to be retroperitoneal.

Unlike the pericardium and pleurae, which smoothly cover the heart and lungs, the peritoneum contains large folds that weave between the viscera. The folds bind the organs to each other and to the walls of the abdominal cavity. They also contain blood vessels, lymphatic vessels, and nerves that supply the abdominal organs.

The greater omentum, the largest peritoneal fold, drapes over the transverse colon and coils of the small intestine like a “fatty apron.” Because the greater omentum is a double sheet that folds back upon itself, it is a four-layered structure. From attachments along the stomach and duodenum, the greater omentum extends downward anterior to the small intestine, then turns and extends upward and attaches to the transverse colon. The greater omentum normally contains a considerable amount of adipose tissue. Its adipose tissue content can greatly expand with weight gain, giving rise to the characteristic “beer belly” seen in some overweight individuals. The many lymph nodes of the greater omentum contribute macrophages and antibody-producing plasma cells that help combat and contain infections of the GI tract.

The falciform ligament attaches the liver to the anterior abdominal wall and diaphragm. The liver is the only digestive organ that is attached to the anterior abdominal wall.

The lesser omentum arises as two folds in the serosa of the stomach and duodenum, and it suspends the stomach and duodenum from the liver. It contains some lymph nodes.

Another fold of the peritoneum, called the mesentery, is fan-shaped and binds the small intestine to the posterior abdominal wall. It extends from the posterior abdominal wall to wrap around the small intestine and then returns to its origin, forming a double mix and moving the luminal contents along the tract. The peritoneum is the largest serous membrane in the body.

Peritonitis

Peritonitis is an acute inflammation of the peritoneum. A common cause of the condition is contamination of the peritoneum by infectious microbes, which can result from accidental or surgical wounds in the abdominal wall, or from perforation or rupture of abdominal organs. If, for example, bacteria gain access to the peritoneal cavity through an intestinal perforation or rupture of the appendix, they can produce an acute, life-threatening form of peritonitis. A less serious form of peritonitis can result from the continual contact of inflamed peritoneal surfaces.

The mouth, also referred to as the oral or buccal cavity, is formed by the cheeks, hard and soft palates, and tongue. Forming the lateral walls of the oral cavity are the cheeks—muscular structures covered externally by skin and internally by nonkeratinized stratified squamous epithelium. The anterior portions of the cheeks end at the lips.

The lips or labia are fleshy folds surrounding the opening of the mouth. They are covered externally by skin and internally by a mucous membrane. There is a transition zone where the two kinds of covering tissue meet. This portion of the lips is nonkeratinized, and the color of the blood in the underlying blood vessels is visible through the transparent surface layer. The inner surface of each lip is attached to its corresponding gum by a midline fold of mucous membrane called the *labial frenulum*.

The *orbicularis oris* muscle and connective tissue lie between the skin and the mucous membrane of the oral cavity. During chewing, contraction of the buccinator muscles in the cheeks and *orbicularis oris* muscle in the lips helps keep food between the upper and lower teeth.

The vestibule of the oral cavity is a space bounded externally by the cheeks and lips and internally by the gums and teeth. The oral cavity proper is a space that extends from the gums and teeth to the fauces, the opening between the oral cavity and the pharynx or throat.

The hard palate—the anterior portion of the roof of the mouth—is formed by the maxillae and palatine bones, is covered by mucous membrane, and forms a bony partition between the oral and nasal cavities. The soft palate, which forms the posterior portion of the roof of the mouth, is an arch-shaped muscular partition between the oropharynx and nasopharynx that is lined by mucous membrane.

Hanging from the free border of the soft palate is a conical muscular process called the uvula. During swallowing, the soft palate and uvula are drawn superiorly, closing off the nasopharynx and preventing swallowed foods and liquids from entering the nasal cavity. Lateral to the base of the uvula are two muscular folds that run down the lateral sides of the soft palate: Anteriorly, the palatoglossal arch extends to the side of the base of the tongue; posteriorly, the palatopharyngeal arch extends to the side of the pharynx. The palatine tonsils are situated between the arches, and the lingual tonsils are situated at the base of the tongue. At the posterior border of the soft palate, the mouth opens into the oropharynx through the fauces.

Salivary Glands

A salivary gland is any cell or organ that releases a secretion called saliva into the oral cavity. Ordinarily, just enough saliva is secreted to keep the mucous membranes of the mouth and pharynx moist and to cleanse the mouth and teeth. When food enters the mouth, however, secretion of saliva increases, and it lubricates, dissolves, and begins the chemical breakdown of the food.

The mucous membrane of the mouth and tongue contains many small salivary glands that open directly, or indirectly via short ducts, to the oral cavity. These glands include labial, buccal, and palatal glands in the lips, cheeks, and palate, respectively, and lingual glands in the tongue, all of which make a small contribution to saliva.

However, most saliva is secreted by the major salivary glands, which lie beyond the oral mucosa. Their secretions empty into ducts that lead to the oral cavity. There are three pairs of major salivary glands: the parotid, submandibular, and sublingual glands. The parotid glands are located inferior and anterior to the ears, between the skin and the masseter muscle. Each secretes saliva into the oral cavity via a parotid

duct that pierces the buccinator muscle to open into the vestibule opposite the second maxillary (upper) molar tooth. The submandibular glands are found beneath the base of the tongue in the posterior part of the floor of the mouth. Their ducts, the submandibular ducts, run under the mucosa on either side of the midline of the floor of the mouth and enter the oral cavity proper lateral to the lingual frenulum. The sublingual glands are superior to the submandibular glands. Their ducts, the lesser sublingual ducts, open into the floor of the mouth in the oral cavity proper.

Composition and Functions of Saliva

Chemically, saliva is 99.5% water and 0.5% solutes. Among the solutes are ions, including sodium, potassium, chloride, bicarbonate, and phosphate. Also present are some dissolved gases and various organic substances, including urea and uric acid, mucus, immunoglobulin A, the bacteriolytic enzyme lysozyme and salivary amylase, a digestive enzyme that acts on starch.

Each major salivary gland supplies different proportions of ingredients to saliva. The parotid glands contain cells that secrete a serous liquid containing salivary amylase. Because the submandibular glands contain cells similar to those found in the parotid glands, plus some mucous cells, they secrete a fluid that contains amylase but is thickened with mucus. The sublingual glands contain mostly mucous cells, so they secrete a much thicker fluid that contributes only a small amount of amylase to the saliva.

The water in saliva provides a medium for dissolving foods so that they can be tasted and digestive reactions can begin. Chloride ions in the saliva activate salivary amylase. Bicarbonate and phosphate ions buffer acidic foods that enter the mouth; as a result, saliva is only slightly acidic (pH 6.35–6.85). Urea and uric acid are found in saliva because salivary glands (like the sweat glands of the skin) help remove waste molecules from the body. Mucus lubricates the food so it can easily be moved about in the mouth, formed into a ball, and swallowed. Immunoglobulin A (IgA) is a secreted type of antibody that prevents attachment of microbes so they cannot penetrate the epithelium. The enzyme lysozyme kills bacteria. Even though these substances help protect the mucous membrane from infection and the teeth from decay, they are not present in large enough quantities to eliminate all oral bacteria.

Tongue

The tongue is an accessory digestive organ composed of skeletal muscle covered with mucous membrane. Together with its associated muscles, it forms the floor of the oral cavity. The tongue is divided into symmetrical lateral halves by a median septum that extends its entire length, and it is attached inferiorly to the hyoid bone, styloid process of the temporal bone, and mandible. Each half of the tongue consists of an identical complement of extrinsic and intrinsic muscles.

The extrinsic muscles of the tongue, which originate outside the tongue and insert into connective tissues in the tongue, include the hyoglossus, genioglossus, and styloglossus muscles. The extrinsic muscles move the tongue from side to side and in and out to maneuver food for chewing, shape the food into a rounded mass, and force

the food to the back of the mouth for swallowing. They also form the floor of the mouth and hold the tongue in position. The intrinsic muscles originate in and insert into connective tissue within the tongue. They alter the shape and size of the tongue for speech and swallowing. The intrinsic muscles include the longitudinalis superior, longitudinalis inferior, transversus linguae, and verticalis linguae muscles. The lingual frenulum, a fold of mucous membrane in the midline of the undersurface of the tongue, is attached to the floor of the mouth and aids in limiting the movement of the tongue posteriorly. If a person's lingual frenulum is abnormally short or rigid—a condition called ankyloglossia—then eating and speaking are impaired such that the person is said to be “tongue-tied.”

The dorsum (upper surface) and lateral surfaces of the tongue are covered with papillae (projections of the *lamina propria* covered with keratinized epithelium). Many papillae contain taste buds, the receptors for taste. Fungiform papillae are mushroomlike elevations distributed among the filiform papillae that are more numerous near the tip of the tongue. They appear as red dots on the surface of the tongue, and most of them contain taste buds. Vallate papillae are arranged in an inverted V shape on the posterior surface of the tongue; all of them contain taste buds. Foliate papillae are located in small trenches on the lateral margins of the tongue but most of their taste buds degenerate in early childhood. Filiform papillae are pointed, threadlike projections distributed in parallel rows over the anterior two-thirds of the tongue. Although filiform papillae lack taste buds, they contain receptors for touch and increase friction between the tongue and food, making it easier for the tongue to move food in the oral cavity. Lingual glands in the *lamina propria* secrete both mucus and a watery serous fluid that contains the enzyme lingual lipase, which acts on triglycerides.

PHARYNX

When food is first swallowed, it passes from the mouth into the pharynx, a funnel-shaped tube that extends from the internal nares to the esophagus posteriorly and to the larynx anteriorly. The pharynx is composed of skeletal muscle and lined by mucous membrane. Whereas the nasopharynx functions only in respiration, both the oropharynx and laryngopharynx have digestive as well as respiratory functions. Swallowed food passes from the mouth into the oropharynx and laryngopharynx, the muscular contractions of which help propel food into the esophagus and then into the stomach.

The movement of food from the mouth into the stomach is achieved by the act of swallowing, or deglutition. Deglutition is facilitated by saliva and mucus and involves the mouth, pharynx, and esophagus. Swallowing occurs in three stages: (1) the voluntary stage, in which the bolus is passed into the oropharynx; (2) the pharyngeal stage, which is the involuntary passage of the bolus through the pharynx into the esophagus; and (3) the esophageal stage, which is the involuntary passage of the bolus through the esophagus into the stomach.

Swallowing starts when the bolus is forced to the back of the oral cavity and into the oropharynx by the movement of the tongue upward and backward against the palate; these actions constitute the voluntary stage of swallowing. With the passage

of the bolus into the oropharynx, the involuntary pharyngeal stage of swallowing begins. The respiratory passageways close, and breathing is temporarily interrupted. The bolus stimulates receptors in the oropharynx, which send impulses to the deglutition center in the medulla oblongata and lower pons of the brain stem. The returning impulses cause the soft palate and uvula to move upward to close off the nasopharynx, and the larynx is pulled forward and upward under the tongue. As the larynx rises, the epiglottis moves backward and downward and seals off the rima glottidis. The movement of the larynx also pulls the vocal cords together, further sealing off the respiratory tract, and widens the opening between the laryngopharynx and esophagus. The bolus passes through the laryngopharynx and enters the esophagus in 1 to 2 seconds. The respiratory passageways then reopen, and breathing resumes.

ESOPHAGUS

The esophagus is a collapsible muscular tube that lies posterior to the trachea. It is about 25 cm (10 in.) long. The esophagus begins at the inferior end of the laryngopharynx and passes through the mediastinum anterior to the vertebral column. Then, it pierces the diaphragm through an opening called the esophageal hiatus and ends in the superior portion of the stomach. Sometimes, part of the stomach protrudes above the diaphragm through the esophageal hiatus. This condition is termed hiatal hernia.

Histology of the Esophagus

The mucosa of the esophagus consists of nonkeratinized stratified squamous epithelium, *lamina propria* (areolar connective tissue), and a *muscularis mucosae* (smooth muscle). Near the stomach, the mucosa of the esophagus also contains mucous glands. The stratified squamous epithelium associated with the lips, mouth, tongue, oropharynx, laryngopharynx, and esophagus affords considerable protection against abrasion and wear-and-tear from food particles that are chewed, mixed with secretions, and swallowed. The submucosa contains areolar connective tissue, blood vessels, and mucous glands. The muscularis of the superior third of the esophagus is skeletal muscle; the intermediate third is skeletal and smooth muscle. and the inferior third is smooth muscle. The superficial layer is known as the adventitia, rather than the serosa, because the areolar connective tissue of this layer is not covered by mesothelium and because the connective tissue merges with the connective tissue of surrounding structures of the mediastinum, through which it passes. The adventitia attaches the esophagus to surrounding structures.

The esophagus secretes mucus and transports food into the stomach. It does not produce digestive enzymes, and it does not carry on absorption. The passage of food from the laryngopharynx into the esophagus is regulated at the entrance to the esophagus by a sphincter (a circular band or ring of muscle that is normally contracted) called the upper esophageal sphincter. It consists of the cricopharyngeus muscle attached to the cricoid cartilage. The elevation of the larynx during the

pharyngeal stage of swallowing causes the sphincter to relax, and the bolus enters the esophagus. This sphincter also relaxes during exhalation.

During the esophageal stage of swallowing, peristalsis, a progression of coordinated contractions and relaxations of the circular and longitudinal layers of the muscularis, pushes the food bolus onward. (Peristalsis occurs in other tubular structures, including other parts of the GI tract and the ureters, bile ducts, and uterine tubes; in the esophagus it is controlled by the medulla oblongata.) In the section of the esophagus just superior to the bolus, the circular muscle fibers contract, constricting the esophageal wall and squeezing the bolus toward the stomach. Meanwhile, longitudinal fibers inferior to the bolus also contract, which shortens this inferior section and pushes its walls outward so it can receive the bolus. The contractions are repeated in a wave that pushes the food toward the stomach. Mucus secreted by esophageal glands lubricates the bolus and reduces friction. The passage of solid or semisolid food from the mouth to the stomach takes 4 to 8 seconds; very soft foods and liquids pass through in about 1 second.

Just superior to the diaphragm, the esophagus narrows slightly due to a maintained contraction of the muscularis at the lowest part of the esophagus. This physiological sphincter, known as the lower esophageal sphincter, relaxes during swallowing and thus allows the bolus to pass from the esophagus into the stomach.

Anatomy of the Stomach

The stomach has four main regions: the cardia, fundus, body, and pylorus (Figure 1.2). The cardia surrounds the superior opening of the stomach. The rounded portion superior to and to the left of the cardia is the fundus. Inferior to the fundus is the large central portion of the stomach, called the body. The region of the stomach that connects to the duodenum is the pylorus; it has two parts—the pyloric antrum, which connects to the body of the stomach, and the pyloric canal, which leads into the duodenum. When the stomach is empty, the mucosa lies in large folds, called

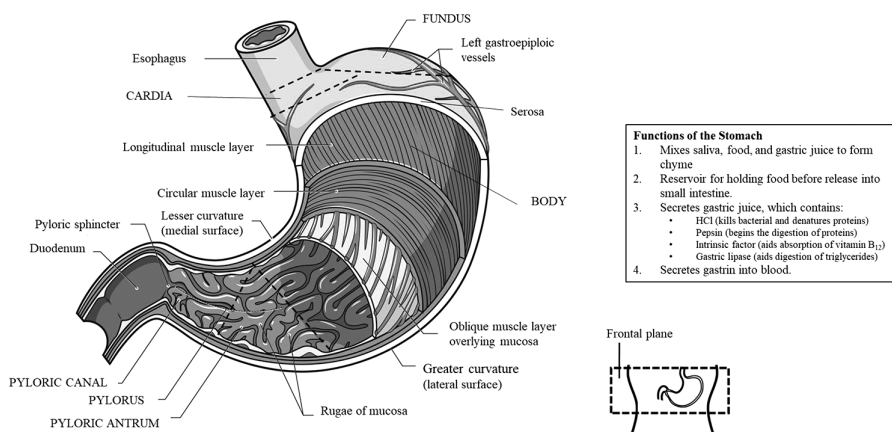


Figure 1.2 Anterior view of regions of stomach. (From Pokusay, A., *The Structure of the Stomach*, Image ID 62703058, Dreamstime.com, http://www.dreamstime.com/alexanderpokusay_info.)

rugae, that can be seen with the unaided eye. The pylorus communicates with the duodenum of the small intestine via a sphincter called the pyloric sphincter. The concave medial border of the stomach is called the lesser curvature, and the convex lateral border is called the greater curvature.

Histology of the Stomach

The stomach wall is composed of the same four basic layers as the rest of the GI tract, with certain modifications. The surface of the mucosa is a layer of simple columnar epithelial cells called surface mucous cells. The mucosa contains a *lamina propria* (areolar connective tissue) and a *muscularis mucosae* (smooth muscle). Epithelial cells extend down into the *lamina propria*, where they form columns of secretory cells called gastric glands that line many narrow channels called gastric pits. Secretions from several gastric glands flow into each gastric pit and then into the lumen of the stomach.

The gastric glands contain three types of exocrine gland cells that secrete their products into the stomach lumen: mucous neck cells, chief cells, and parietal cells. Both surface mucous cells and mucous neck cells secrete mucus. Parietal cells produce hydrochloric acid and the intrinsic factor needed for absorption of vitamin B₁₂. The chief cells secrete pepsinogen and gastric lipase. The secretions of the mucous, parietal, and chief cells form gastric juice, which totals 2000–3000 mL (roughly 2–3 qt.) per day. In addition, gastric glands include a type of enteroendocrine cell, the G cell, which is located mainly in the pyloric antrum and secretes the hormone gastrin into the bloodstream. As we will see shortly, this hormone stimulates several aspects of gastric activity.

Three additional layers lie deep to the mucosa. The submucosa of the stomach is composed of areolar connective tissue. The muscularis has three layers of smooth muscle: an outer longitudinal layer, a middle circular layer, and an inner oblique layer. The oblique layer is limited primarily to the body of the stomach. The serosa is composed of simple squamous epithelium (mesothelium) and areolar connective tissue, and the portion of the serosa covering the stomach is part of the visceral peritoneum. At the lesser curvature of the stomach, the visceral peritoneum extends upward to the liver as the lesser omentum. At the greater curvature of the stomach, the visceral peritoneum continues downward as the greater omentum and drapes over the intestines.

Anatomy of the Small Intestine

The small intestine is divided into three regions. The duodenum, the shortest region, is retroperitoneal. It starts at the pyloric sphincter of the stomach and extends about 25 cm until it merges with the jejunum. The jejunum is about 1 m long and extends to the ileum. Jejunum means “empty,” which is how it is found at death. The final and longest region of the small intestine, the ileum, measures about 2 m and joins the large intestine at the ileocecal sphincter.

Projections called circular folds are 10 mm (0.4 in.) permanent ridges in the mucosa. The circular folds begin near the proximal portion of the duodenum and end

at about the midportion of the ileum; some extend all the way around the circumference of the intestine, and others extend only part of the way around. They enhance absorption by increasing surface area and causing the chyme to spiral, rather than move in a straight line, as it passes through the small intestine.

Histology of the Small Intestine

Even though the wall of the small intestine is composed of the same four coats that make up most of the GI tract, special features of both the mucosa and the submucosa facilitate the process of digestion and absorption. The mucosa forms a series of fingerlike villi, projections that are 0.5–1 mm long. The large number of villi (20–40 mm²) vastly increases the surface area of the epithelium available for absorption and digestion and gives the intestinal mucosa a velvety appearance. Each villus has a core of *lamina propria*; embedded in this connective tissue are an arteriole, a venule, a blood capillary network, and a lacteal, which is a lymphatic capillary. Nutrients absorbed by the epithelial cells covering the villus pass through the wall of a capillary or a lacteal to enter blood or lymph, respectively.

The epithelium of the mucosa consists of simple columnar epithelium that contains absorptive cells, goblet cells, enteroendocrine cells, and Paneth cells. The apical membrane of absorptive cells features microvilli; each microvillus is 10 mm long cylindrical, membrane-covered projection that contains a bundle of 20–30 actin filaments.

The mucosa contains many deep crevices lined with glandular epithelium. Cells lining the crevices form the intestinal glands (crypts of Lieberkühn) and secrete intestinal juice. Many of the epithelial cells in the mucosa are goblet cells, which secrete mucus. Paneth cells, found in the deepest parts of the intestinal glands, secrete lysozyme, a bactericidal enzyme, and are capable of phagocytosis. They may have a role in regulating the microbial population in the intestines. Three types of enteroendocrine cells, also in the deepest part of the intestinal glands, secrete hormones: secretin (by S cells), cholecystokinin (by CCK cells), and glucose-dependent insulinotropic peptide (by K cells). The *lamina propria* of the small intestine has an abundance of MALT. Solitary lymphatic nodules are most numerous in the distal part of the ileum. Groups of lymphatic nodules referred to as aggregated lymphatic follicles (Peyer's patches) are also present in the ileum. The *muscularis mucosae* consists of smooth muscle. The submucosa of the duodenum contains duodenal (Brunner's) glands, which secrete an alkaline mucus that helps neutralize gastric acid in the chyme. Sometimes the lymphatic tissue of the *lamina propria* extends through the *muscularis mucosae* into the submucosa.

The muscularis of the small intestine consists of two layers of smooth muscle. The outer, thinner layer contains longitudinal fibers; the inner, thicker layer contains circular fibers. Except for a major portion of the duodenum, the serosa (or visceral peritoneum) completely surrounds the small intestine.

The large intestine is the terminal portion of the GI tract and is divided into four principal regions. The overall functions of the large intestine are the completion of absorption, the production of certain vitamins, the formation of feces, and the expulsion of feces from the body.

Anatomy of the Large Intestine

The large intestine, which is about 1.5 m long and 6.5 cm in diameter, extends from the ileum to the anus. It is attached to the posterior abdominal wall by its mesocolon, which is a double layer of peritoneum. Structurally, the four major regions of the large intestine are the cecum, colon, rectum, and anal canal.

The opening from the ileum into the large intestine is guarded by a fold of mucous membrane called the ileocecal sphincter, which allows materials from the small intestine to pass into the large intestine. Hanging inferior to the ileocecal valve is the cecum, a blind pouch about 6 cm long. Attached to the cecum is a twisted, coiled tube, measuring about 8 cm in length, called the appendix or vermiform appendix. The mesentery of the appendix, called the mesoappendix, attaches the appendix to the inferior part of the mesentery ileum.

The open end of the cecum merges with a long tube called the colon, which is divided into ascending, transverse, descending, and sigmoid portions. Both the ascending and descending colon are retroperitoneal, whereas the transverse and sigmoid colon are not. The ascending colon ascends on the right side of the abdomen, reaches the inferior surface of the liver, and turns abruptly to the left to form the right colic flexure. The colon continues across the abdomen to the left side as the transverse colon. It curves beneath the inferior end of the spleen on the left side as the left colic flexure and passes inferiorly to the level of the iliac crest as the descending colon. The sigmoid colon begins near the left iliac crest, projects medially to the midline, and terminates at the rectum at about the level of the third sacral vertebra.

The rectum, the last 20 cm of the GI tract, lies anterior to the sacrum and coccyx. The terminal 2–3 cm of the rectum is called the anal canal. The mucous membrane of the anal canal is arranged in longitudinal folds called anal columns that contain a network of arteries and veins. The opening of the anal canal to the exterior, called the anus, is guarded by an internal anal sphincter of smooth muscle (involuntary) and an external anal sphincter of skeletal muscle. Normally these sphincters keep the anus closed except during the elimination of feces.

Histology of the Large Intestine

The wall of the large intestine differs from that of the small intestine in several respects. No villi or permanent circular folds are found in the mucosa, which consists of simple columnar epithelium, *lamina propria* (areolar connective tissue), and *muscularis mucosae*. The epithelium contains mostly absorptive and goblet cells. The absorptive cells function primarily in water absorption, whereas the goblet cells secrete mucus that lubricates the passage of the colonic contents. Both absorptive and goblet cells are located in long, straight, tubular intestinal glands that extend the full thickness of the mucosa. Solitary lymphatic nodules are also found in the *lamina propria* of the mucosa and may extend through the *muscularis mucosae* into the submucosa. The submucosa of the large intestine is similar to that found in the rest of the GI tract. The muscularis consists of an external layer of longitudinal smooth muscle and an internal layer of circular smooth muscle.

FUNCTION

Several minutes after food enters the stomach, gentle, rippling, peristaltic movements called mixing waves pass over the stomach every 15 to 25 seconds. These waves macerate food, mix it with secretions of the gastric glands, and reduce it to a soupy liquid called chyme. Few mixing waves are observed in the fundus, which primarily has a storage function. As digestion proceeds in the stomach, more vigorous mixing waves begin at the body of the stomach and intensify as they reach the pylorus. The pyloric sphincter normally remains almost, but not completely, closed. As food reaches the pylorus, each mixing wave forces several milliliters of chyme into the duodenum through the pyloric sphincter. Most of the chyme is forced back into the body of the stomach, where mixing continues. The next wave pushes the chyme forward again and forces a little more into the duodenum. These forward and backward movements of the gastric contents are responsible for most mixing in the stomach.

Mechanical and Chemical Digestion in the Mouth

Mechanical digestion in the mouth results from chewing, or mastication (mas-ti-KA-shun = to chew), in which food is manipulated by the tongue, ground by the teeth, and mixed with saliva. As a result, the food is reduced to a soft, flexible, easily swallowed mass called a bolus (= lump). Food molecules begin to dissolve in the water in saliva, an important activity because enzymes can react with food molecules in a liquid medium only.

Two enzymes, salivary amylase and lingual lipase, contribute to chemical digestion in the mouth. Salivary amylase initiates the breakdown of starch. Dietary carbohydrates are either monosaccharide and disaccharide sugars or complex polysaccharides.

Foods may remain in the fundus for about an hour without becoming mixed with gastric juice. During this time, digestion by salivary amylase continues. Soon, however, the churning action mixes chyme with acidic gastric juice, inactivating salivary amylase and activating lingual lipase, which starts to digest triglycerides into fatty acids and triglycerides.

Even though parietal cells secrete hydrogen ions (H^+) and chloride ions (Cl^-) separately into the stomach lumen, the net effect is secretion of hydrochloric acid (HCL). Proton pumps powered by H^+/K^+ ATPases actively transport H^+ into the lumen while bringing potassium ions (K^+) into the cell (Figure 1.3). At the same time, Cl^- and K^+ diffuse out through Cl and K^+ channels in the apical membrane (next to the lumen). The enzyme carbonic anhydrase, which is especially plentiful in parietal cells, catalyzes the formation of carbonic acid (H_2CO_3) from water (H_2O) and carbon dioxide (CO_2). As carbonic acid dissociates, it provides a ready source of H^+ for the proton pumps but also generates bicarbonate ions (HCOO^-). As HCO_3^- builds up in the cytosol, it exits the parietal cell in exchange for Cl^- via $\text{Cl}^-/\text{HCO}_3^-$ antiporters in

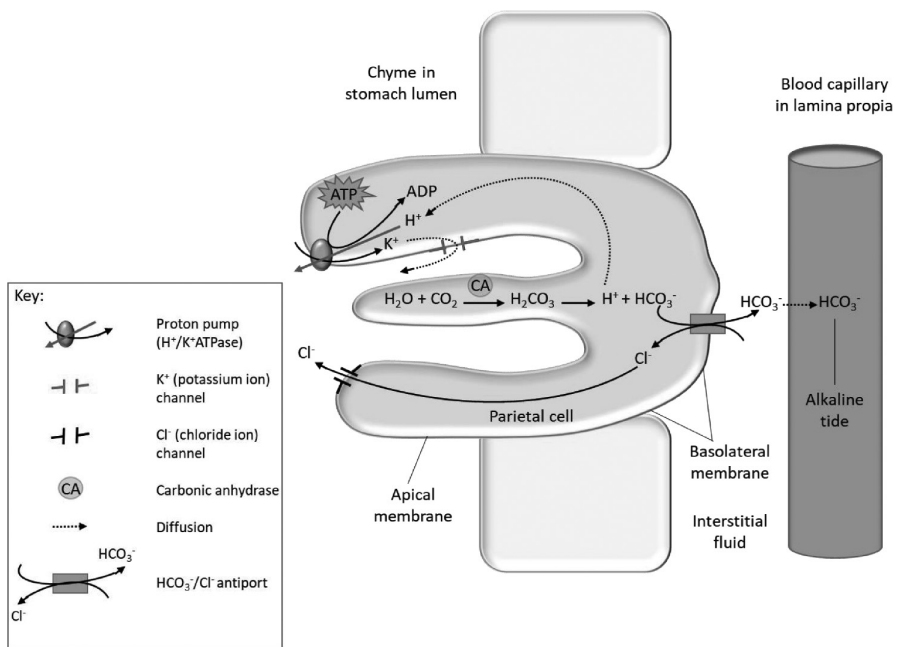


Figure 1.3 Transport of hydrogen ions into lumen and potassium ions into cells.

the basolateral membrane (next to the *lamina propria*). HCO₃⁻ diffuses into nearby blood capillaries. This “alkaline tide” of bicarbonate ions entering the bloodstream after a meal may be large enough to slightly elevate blood pH and make urine more alkaline.

The strongly acidic fluid of the stomach kills many microbes in food, and HCL partially denatures proteins in food and stimulates the secretion of hormones that promote the flow of bile and pancreatic juice. Enzymatic digestion of proteins also begins in the stomach. The only proteolytic enzyme in the stomach is pepsin, which is secreted by chief cells. Because pepsin breaks certain peptide bonds between the amino acids making up proteins, a protein chain of many amino acids is broken down into smaller peptide fragments. Pepsin is most effective in the very acidic environment of the stomach (pH 2); it becomes inactive at higher pH.

Note that pepsin is secreted in an inactive form called pepsinogen; in this form, it cannot digest the proteins in the chief cells that produce it. Pepsinogen is not converted into active pepsin until it comes in contact with active pepsin molecules or hydrochloric acid secreted by parietal cells. The stomach epithelial cells are also protected from gastric juices by a 1–3 mm thick layer of alkaline mucus secreted by surface mucous cells and mucous neck cells.

Another enzyme of the stomach is gastric lipase, which splits the short-chain triglycerides in fat molecules found in milk into fatty acids and monoglycerides. This enzyme, which has a limited role in the adult stomach, operates best at a pH of 5–6. More important than either lingual lipase or gastric lipase is pancreatic lipase, an enzyme secreted by the pancreas into the small intestine.

Only a small amount of absorption occurs in the stomach because its epithelial cells are impermeable to most materials. However, mucous cells of the stomach absorb some water, ions, and short-chain fatty acids, as well as certain drugs (especially aspirin and other “NSAIDs”) and alcohol.

Regulation of Gastric Secretion and Motility

Both neural and hormonal mechanisms control the secretion of gastric juice and the contraction of smooth muscle in the stomach wall. Events in gastric digestion occur in three overlapping phases: the cephalic, gastric, and intestinal phases.

Cephalic Phase

The cephalic phase of gastric digestion consists of reflexes initiated by sensory receptors in the head. Even before food enters the stomach, the sight, smell, taste, or thought of food initiates this reflex. The cerebral cortex and the feeding center in the hypothalamus send nerve impulses to the medulla oblongata. The medulla then transmits impulses to parasympathetic preganglionic neurons in the vagus nerves, which stimulate parasympathetic postganglionic neurons in the submucosal plexus. In turn, impulses from parasympathetic postganglionic neurons stimulate the gastric glands to secrete pepsinogen, hydrochloric acid, and mucus into stomach chyme, and gastrin into the blood. Impulses from parasympathetic neurons also increase stomach motility. Emotions such as anger, fear, and anxiety may slow digestion in the stomach because they stimulate the sympathetic nervous system, which inhibits gastric activity.

Gastric Phase

Once food reaches the stomach, sensory receptors in the stomach initiate both neural and hormonal mechanisms to ensure that gastric secretion and motility continue. This is the gastric phase of gastric digestion. Food of any kind distends (stretches) the stomach and stimulates stretch receptors in its walls. Chemoreceptors in the stomach monitor the pH of the stomach chyme. When the stomach walls are distended or pH increases because proteins have entered the stomach and glands have secreted some of the stomach acid, the stretch receptors and chemoreceptors are activated, and a neural negative feedback loop is set in motion. From the stretch receptors and chemoreceptors, nerve impulses propagate to the submucosal plexus, where they activate parasympathetic and enteric neurons. The resulting nerve impulses cause waves of peristalsis and continue to stimulate the flow of gastric juice from parietal cells, chief cells, and mucous cells.

The peristaltic waves mix the food with gastric juice, and when the waves become strong enough, a small quantity of chyme (about 10–15 mL) squirts past the pyloric sphincter into the duodenum. As the pH of the stomach chyme becomes more acidic again and the stomach walls are less distended because chyme has passed into the small intestine, a negative feedback cycle suppresses secretion of gastric juice.

During the cephalic and gastric phases, digestion in the stomach is stimulated whereas during the intestinal phase, gastric juice secretion and gastric peristalsis are inhibited.

Hormonal negative feedback also regulates gastric secretions, during the gastric phase. Partially digested proteins buffer H^+ , thus increasing pH, and ingested food distends the stomach. Chemoreceptors and stretch receptors monitoring these changes stimulate parasympathetic neurons which release acetylcholine. In turn, acetylcholine stimulates secretion of the hormone gastrin by G cells, enteroendocrine cells in the mucosa of the pyloric antrum. (A small amount of gastrin is also secreted by enteroendocrine cells in the small intestine; Additionally, some chemicals in food directly stimulates gastrin release.) Gastrin enters the bloodstream and finally reaches its target cells, the gastric glands.

Gastrin stimulates growth of the gastric glands and secretion of large amounts of gastric juice. It also strengthens contraction of the lower esophageal sphincter, increases motility of the stomach, and relaxes the pyloric and ileocecal sphincter (described later). Gastrin secretion is inhibited when the pH of gastric juice drops below 2.0 and is stimulated when the pH rises. This negative feedback mechanism helps provide an optimal low pH for the functioning of pepsin, the killing of microbes, and the denaturing of proteins in the stomach.

Acetylcholine released by parasympathetic neurons and gastrin secreted by G cells stimulate parietal cells to secrete more HCl in the presence of histamine. In other words, histamine, a paracrine substance that is released by mast cells in the *lamina propria* and acts on nearby parietal cells, acts synergistically with acetylcholine and gastrin to enhance their effects. Receptors for all three substances are present in the plasma membrane of parietal cells. The histamine receptors on parietal cells are called H_2 receptors; they mediate different responses than do the H_1 receptors involved in allergic responses.

Intestinal Phase

The intestinal phase of gastric digestion is due to activation of receptors in the small intestine. Whereas reflexes initiated during the cephalic and gastric phases stimulate stomach secretory activity and motility, those occurring during the intestinal phase have inhibitory effects that slow the exit of chyme from the stomach and prevent overloading of the duodenum with more chyme than it can handle. In addition, responses occurring during the intestinal phase promote the continued digestion of foods that have reached the small intestine. When chyme containing fatty acids and glucose leaves the stomach and enters the small intestine, it triggers enteroendocrine cells in the small intestinal mucosa to release into the blood two hormones that affect the stomach—secretin and cholecystokinin (CCK).

Regulation of Gastric Emptying

Gastric emptying, the periodic release of chyme from the stomach into the duodenum, is regulated by both neural and hormonal reflexes, as follows:

1. Stimuli such as distention of the stomach and the presence of partially digested proteins, alcohol, and caffeine initiate gastric emptying.
2. These stimuli increase the secretion of gastrin and generate parasympathetic impulses in the vagus nerves.
3. Gastrin and nerve impulses stimulate contraction of the lower esophageal sphincter, increase motility of the stomach, and relax the pyloric sphincter.
4. The net effect of these actions is gastric emptying.

Neural and hormonal reflexes also help ensure that the stomach does not release more chyme than the small intestine can process. The neural reflex called the enterogastric reflex and the hormone cholecystokinin inhibit gastric emptying as follows:

1. Stimuli such as distention of the duodenum and the presence of fatty acids, glucose, and partially digested proteins in the duodenal chyme inhibit gastric emptying.
2. These stimuli then initiate the enterogastric reflex. Nerve impulses propagate from the duodenum to the medulla oblongata, where they inhibit parasympathetic stimulation and stimulate sympathetic activity in the stomach. The same stimuli also increase secretion of cholecystokinin.
3. Increased sympathetic impulses and cholecystokinin both decrease gastric motility.
4. The net effect of these actions is inhibition of gastric emptying.

Within 2 to 4 hours after eating a meal, the stomach has emptied its contents into the duodenum. Foods rich in carbohydrate spend the least time in the stomach; high-protein foods remain somewhat longer, and emptying is slowest after a fat-laden meal containing large amounts of triglycerides. The reason for slower emptying after eating triglycerides is that fatty acids in chyme stimulate release of cholecystokinin, which slows stomach emptying.

Vomiting or emesis is the forcible expulsion of the contents of the upper tract (stomach and sometimes duodenum) through the mouth. The strongest stimuli for vomiting are irritation and distension of the stomach; other stimuli include unpleasant sights, general anesthesia, dizziness, and certain drugs such as morphine and derivatives of digitalis. Nerve impulses are transmitted to the vomiting center in the medulla oblongata, and returning impulses propagate to the upper tract organs, diaphragm, and abdominal muscles. Vomiting involves squeezing the stomach between the diaphragm and abdominal muscles and expelling the contents through open esophageal sphincters. Prolonged vomiting, especially in infants and elderly people, can be serious because the loss of acidic gastric juice can lead to alkalosis (higher than normal blood pH).

From the stomach, chyme passes into the small intestine. Because chemical digestion in the small intestine depends on activities of the pancreas, liver, and gallbladder, we first consider the activities of these accessory digestive organs and their contributions to digestion in the small intestine.

Role and Composition of Bile

Each day, hepatocytes in the liver secrete 800–1000 mL (about one qt.) of bile, a yellow, brownish, or olive-green liquid. It has a pH of 7.6–8.6 and consists mostly of water and bile acids, bile salts, cholesterol, a phospholipid called lecithin, bile pigments, and several ions.

Bile is partially an excretory product and partially a digestive secretion. Bile salts, which are sodium salts and potassium salts of bile acids (mostly cholic acid and chenodeoxycholic acid), play a role in emulsification, the breakdown of large lipid globules into a suspension of droplets about 1 μm in diameter, and in the absorption of lipids following their digestion. The tiny lipid droplets present a very large surface area that allows pancreatic lipase to more rapidly accomplish digestion of triglycerides. Cholesterol is made soluble in bile by bile salts and lecithin.

The principal bile pigment is conjugated bilirubin. The phagocytosis of aged red blood cells liberates iron, globin, and bilirubin (derived from heme). The iron and globin are recycled, and some of the bilirubin is converted to conjugated bilirubin, in which the bilirubin is attached to glucuronic acid molecules. Conjugated bilirubin is then secreted into the bile and is eventually broken down in the intestine. One of its breakdown products—stercobilin—gives feces their normal brown color.

Regulation of Bile Secretion

After they have served their function as emulsifying agents, most bile salts are reabsorbed by active transport in the final segment of the small intestine (ileum) and enter portal blood flowing toward the liver. Although hepatocytes continually release bile, they increase production and secretion when the portal blood contains more bile acids; thus, as digestion and absorption continue in the small intestine, bile release increases. Between meals, after most absorption has occurred, bile flows into the gallbladder for storage because the sphincter of the hepatopancreatic ampulla closes off the entrance to the duodenum. After a meal, several neural and hormonal stimuli promote production and release of bile:

1. Parasympathetic impulses propagate along axons of the vagus nerve and stimulate the liver to increase bile production.
2. Fatty acids and amino acids in chyme entering the duodenum stimulate some duodenal enteroendocrine cells to secrete the hormone cholecystokinin into the blood. Acidic chyme entering the duodenum stimulates other enteroendocrine cells to secrete the hormone secretin into the blood.

- 3. CCK causes contraction of the wall of the gallbladder, which squeezes stored bile out of the gallbladder into the cystic duct and through the common bile duct. cholecystokinin also causes relaxation of the sphincter of the hepatopancreatic ampulla, which allows bile to flow into the duodenum.
- 4. Secretin, which stimulates secretion of pancreatic juice that is rich in HCO_3 , also stimulates the secretion of HCO_3^- by hepatocytes into bile.

Stretching of the stomach as it receives food and the buffering of gastric acids by proteins in food trigger the release of gastrin. Gastrin, in turn, promotes secretion of gastric juice and increases gastric motility so that ingested food becomes well mixed into a thick, soupy chyme. Reflux of the acidic chyme into the esophagus is prevented by tonic contraction of the lower esophageal sphincter, which is enhanced by gastrin.

The major stimulus for secretion of secretin is acidic chyme (high concentration of H^+) entering the small intestine. In turn, secretin promotes secretion of bicarbonate ions HCO_3 into pancreatic juice and bile. HCO_3 acts to buffer (“soak up”) excess HCO_3 . Besides these major effects, secretin inhibits secretion of gastric juice, promotes normal growth and maintenance of the pancreas, and enhances the effects of CCK. Overall, secretin causes buffering of acid in chyme that reaches the duodenum and slows production of acid in the stomach. [Table 1.1](#) summarizes the hormones which control digestion.

Table 1.1 Major Hormones That Control Digestion

Hormone	Stimulus and Site of Secretion	Actions
Gastrin	Distension of stomach, partially digested proteins and caffeine in stomach, and high pH of stomach chyme stimulate gastrin secretion by enteroendocrine G cells, located mainly in the mucosa of pyloric antrum of stomach.	Major effects: Promotes secretion of gastric juice, increases gastric motility, and promotes growth of gastric mucosa. Minor effects: Constricts lower esophageal sphincter; relaxes pyloric sphincter and ileocecal sphincter.
Secretin	Acidic (high H^+ level) chyme that enters the small intestine stimulates secretion of secretin by enteroendocrine S cells in the mucosa of the duodenum.	Major effects: Stimulates secretion of pancreatic juice and bile that are rich in HCO_3 (bicarbonate ions). Minor effects: Inhibits secretion of gastric juice, promotes normal growth and maintenance of the pancreas, and enhances effects of CCK.
Cholecystokinin (CCK)	Partially digested proteins (amino acids), triglycerides, and fatty acids that enter the small intestine stimulate secretion of CCK by enteroendocrine CCK cells in the mucosa of the small intestine; CCK is also released in the brain.	Major effects: Stimulates secretion of pancreatic juice rich in digestive enzymes, causes ejection of bile from the gallbladder and opening of the sphincter of the hepatopancreatic ampulla (sphincter of Oddi), and induces satiety (feeling full to satisfaction). Minor effects: Inhibits gastric emptying, promotes normal growth and maintenance of the pancreas, and enhances effects of secretin.

Amino acids from partially digested proteins and fatty acids from partially digested triglycerides stimulate secretion of cholecystokinin by enteroendocrine cells in the mucosa of the small intestine. CCK stimulates secretion of pancreatic juice that is rich in digestive enzymes and ejection of bile into the duodenum, slows gastric emptying by promoting contraction of the pyloric sphincter, and produces satiety (feeling full to satisfaction) by acting on the hypothalamus in the brain. Like secretin, CCK promotes normal growth and maintenance of the pancreas; it also enhances the effects of secretin.

Besides these three hormones, at least ten other so-called “gut hormones” are secreted by and have effects on the GI tract. They include motilin, substance P, and bombesin, which stimulate motility of the intestines; vasoactive intestinal polypeptide (VIP), which stimulates secretion of ions and water by the intestines and inhibits gastric acid secretion; gastrin-releasing peptide, which stimulates release of gastrin; and somatostatin, which inhibits gastrin release. Some of these hormones are thought to act as local hormones, whereas others are secreted into the blood or even into the lumen of the GI tract. The physiological roles of these and other gut hormones are still under investigation.

Now that we have examined the sources of the main digestive enzymes and hormones, we can proceed to the next part of the tract and pick up the story of digestion and absorption. The major events of digestion and absorption occur in a long tube called the small intestine. Because most digestion and absorption of nutrients occur in the small intestine, its structure is specially adapted for these functions. Its length alone provides a large surface area for digestion and absorption, and that area is further increased by circular folds, villi, and microvilli. The small intestine begins at the pyloric sphincter of the stomach, coils through the central and inferior part of the abdominal cavity, and eventually opens into the large intestine. It averages 2.5 cm in diameter; its length is about 3 m in a living person and about 6.5 m in a cadaver due to the loss of smooth muscle tone after death.

Role of Intestinal Juice and Brush-Border Enzymes

Intestinal juice is a clear yellow fluid secreted in amounts of 1–2 L a day. It contains water and mucus and is slightly alkaline (pH 7.6). Together, pancreatic and intestinal juices provide a liquid medium that aids the absorption of substances from chyme as they come in contact with the microvilli. The absorptive epithelial cells synthesize several digestive enzymes, called brush-border enzymes, and insert them in the plasma membrane of the microvilli. Thus, some enzymatic digestion occurs at the surface of the epithelial cells that line the villi, rather than in the lumen exclusively, as occurs in other parts of the GI tract. Among the brush-border enzymes are four carbohydrate-digesting enzymes called α -dextrinase, maltase, sucrase, and lactase; protein-digesting enzymes called peptidases (aminopeptidase and dipeptidase); and two types of nucleotide-digesting enzymes, nucleosidases, and phosphatases. Also, as cells slough off into the lumen of the small intestine, they break apart and release enzymes that help digest nutrients in the chyme.

Mechanical Digestion in the Small Intestine

The two types of movements of the small intestine—segmentations and a type of peristalsis called migrating motility complexes—are governed mainly by the myenteric plexus. Segmentations are localized, mixing contractions that occur in portions of intestine distended by a large volume of chyme. Segmentations mix chyme with the digestive juices and bring the particles of food into contact with the mucosa for absorption; they do not push the intestinal contents along the tract. A segmentation starts with the contractions of circular muscle fibers in a portion of the small intestine, an action that constricts the intestine into segments. Next, muscle fibers that encircle the middle of each segment also contract, dividing each segment again. Finally, the fibers that first contracted relax, and each small segment unites with an adjoining small segment so that large segments are formed again. As this sequence of events repeats, the chyme sloshes back and forth. Segmentations occur most rapidly in the duodenum, about 12 times per minute, and progressively slow to about 8 times per minute in the ileum. This movement is similar to alternately squeezing the middle and then the ends of a capped tube of toothpaste.

After most of a meal has been absorbed, which lessens distention of the wall of the small intestine, segmentation stops and peristalsis begins. The type of peristalsis that occurs in the small intestine, termed a migrating motility complex (MMC), begins in the lower portion of the stomach and pushes chyme forward along a short stretch of small intestine before dying out. The MMC slowly migrates down the small intestine, reaching the end of the ileum in 90–120 minutes. Then another MMC begins in the stomach. Altogether, chyme remains in the small intestine for 3–5 hours.

Chemical Digestion in the Small Intestine

In the mouth, salivary amylase converts starch (a polysaccharide) to maltose (a disaccharide), maltotriose (a trisaccharide), and α -dextrins (short-chain, branched fragments of starch with five to ten glucose units). In the stomach, pepsin converts proteins to peptides (small fragments of proteins), and lingual and gastric lipases convert some triglycerides into fatty acids, diglycerides, and monoglycerides. Thus, chyme entering the small intestine contains partially digested carbohydrates, proteins, and lipids. The completion of the digestion of carbohydrates, proteins, and lipids is a collective effort of pancreatic juice, bile, and intestinal juice in the small intestine.

Digestion of Carbohydrates

Even though the action of salivary amylase may continue in the stomach for a while, the acidic pH of the stomach destroys salivary amylase and ends its activity. Thus, only a few starches are reduced to maltose by the time chyme leaves the stomach. Those starches not already broken down into maltose, maltotriose, and

α -dextrins are cleaved by pancreatic amylase, an enzyme in pancreatic juice that acts in the small intestine. Although amylase acts on both glycogen and starches, it does not act on another polysaccharide called cellulose, which is an indigestible plant fiber. After amylase has split starch into smaller fragments, a brush-border enzyme called α -dextrinase acts on the resulting α -dextrins, clipping off one glucose unit at a time.

Ingested molecules of sucrose, lactose, and maltose—three disaccharides—are not acted on until they reach the small intestine. Three brush-border enzymes digest the disaccharides into monosaccharides. Sucrase breaks sucrose into a molecule of glucose and a molecule of fructose; lactase digests lactose into a molecule of glucose and a molecule of galactose; and maltase splits maltose and maltotriose into two or three molecules of glucose, respectively. Digestion of carbohydrates ends with the production of monosaccharides, as mechanisms exist for their absorption.

Lactose Intolerance

In some people the mucosal cells of the small intestine fail to produce enough lactase, which is essential for the digestion of lactose. This results in a condition called lactose intolerance, in which undigested lactose in chyme retains fluid in the feces and bacterial fermentation of lactose results in the production of gases. Symptoms of lactose intolerance include diarrhea, gas, bloating, and abdominal cramps after consumption of milk and other dairy products. The severity of symptoms varies from relatively minor to sufficiently serious to require medical attention. Persons with lactose intolerance can take dietary supplements to aid in the digestion of lactose.

Digestion of Proteins

Protein digestion starts in the stomach, where proteins are fragmented into peptides by the action of pepsin. Enzymes in pancreatic juice—trypsin, chymotrypsin, carboxypeptidase, and elastase—continue to break down proteins into peptides. Although all these enzymes convert whole proteins into peptides, their actions differ somewhat because each splits peptide bonds between different amino acids. Trypsin, chymotrypsin, and elastase all cleave the peptide bond between a specific amino acid and its neighbor; carboxypeptidase breaks the peptide bond that attaches the terminal amino acid to the carboxyl (acid) end of the peptide. Protein digestion is completed by two peptidases in the brush border: aminopeptidase and dipeptidase. Aminopeptidase acts on peptides by breaking the peptide bond that attaches the terminal amino acid to the amino end of the peptide. Dipeptidase splits dipeptides into single amino acids.

Digestion of Lipids

The most abundant lipids in the diet are triglycerides, which consist of a molecule of glycerol bonded to three fatty acid molecules. Enzymes that split triglycerides

and phospholipids are called lipases. In adults, most lipid digestion occurs in the small intestine, although some occur in the stomach through the action of lingual and gastric lipases. When chyme enters the small intestine, bile salts emulsify the globules of triglycerides into droplets about 11 μm in diameter, which increases the surface area exposed to pancreatic lipase, another enzyme in pancreatic juice. This enzyme hydrolyzes triglycerides into fatty acids and monoglycerides, the main end products of triglyceride digestion. Pancreatic and gastric lipases remove two of the three fatty acids from glycerol; the third remains attached to the glycerol, forming a monoglyceride.

Digestion of Nucleic Acids

Pancreatic juice contains two nucleases: ribonuclease, which digests RNA, and deoxyribonuclease, which digests DNA. The nucleotides that result from the action of the two nucleases are further digested by brush-border enzymes called nucleosidases and phosphatases into pentoses, phosphates, and nitrogenous bases. These products are absorbed via active transport.

Regulation of Intestinal Secretion and Motility

The most important mechanisms that regulate small intestinal secretion and motility are enteric reflexes that respond to the presence of chyme; vasoactive intestinal polypeptide also stimulates the production of intestinal juice. Segmentation movements depend mainly on intestinal distention, which initiates nerve impulses to the enteric plexuses and the central nervous system. Enteric reflexes and returning parasympathetic impulses from the CNS increase motility; sympathetic impulses decrease intestinal motility. Migrating motility complexes strengthen when most nutrients and water have been absorbed—that is, when the walls of the small intestine are less distended. With more vigorous peristalsis, the chyme moves along toward the large intestine as fast as 10 cm/sec. The first remnants of a meal reach the beginning of the large intestine in about 4 hours.

Absorption in the Small Intestine

All the chemical and mechanical phases of digestion from the mouth through the small intestine are directed toward changing food into forms that can pass through the epithelial cells lining the mucosa and into the underlying blood and lymphatic vessels. These forms are monosaccharides (glucose, fructose, and galactose) from carbohydrates; single amino acids, dipeptides, and tripeptides from proteins; and fatty acids, glycerol, and monoglycerides from triglycerides. Passage of these digested nutrients from the gastrointestinal tract into the blood or lymph is called absorption.

Absorption of materials occurs via diffusion, facilitated diffusion, osmosis, and active transport. About 90% of all absorption of nutrients occurs in the small intestine; the other 10% occurs in the stomach and large intestine. Any undigested or unabsorbed material left in the small intestine passes on to the large intestine.

Absorption of Monosaccharides

All carbohydrates are absorbed as monosaccharides. The capacity of the small intestine to absorb monosaccharides is huge—an estimated 120 g/h. As a result, all dietary carbohydrates that are digested normally are absorbed, leaving only indigestible cellulose and fibers in the feces. Monosaccharides pass from the lumen through the apical membrane via facilitated diffusion or active transport. Fructose, a monosaccharide found in fruits, is transported via facilitated diffusion; glucose and galactose are transported into epithelial cells of the villi via secondary active transport that is coupled to the active transport of Na^+ . The transporter has binding sites for one glucose molecule and two sodium ions; unless all three sites are filled, neither substance is transported. Galactose competes with glucose to ride the same transporter. (Because both Na^+ and glucose or galactose move in the same direction, this is a symporter). The same type of Na^+ glucose symporter reabsorbs filtered blood glucose in the tubules of the kidneys. Monosaccharides then move out of the epithelial cells through their basolateral surfaces via facilitated diffusion and enter the capillaries of the villi.

Absorption of Amino Acids, Dipeptides, and Tripeptides

Most proteins are absorbed as amino acids via active transport processes that occur mainly in the duodenum and jejunum. About half of the absorbed amino acids are present in food, whereas the other half comes from proteins in digestive juices and dead cells that slough off the mucosal surface! Normally, 95%–98% of the protein present in the small intestine is digested and absorbed. Several transporters carry different types of amino acids. Some amino acids enter epithelial cells of the villi via Na^+ -dependent secondary active transport processes that are similar to the glucose transporter; other amino acids are actively transported by themselves. At least one symporter brings in dipeptides and tripeptides together with H^+ ; the peptides then are hydrolyzed to single amino acids inside the epithelial cells. Amino acids move out of the epithelial cells via diffusion and enter capillaries of the villus. Both monosaccharides and amino acids are transported in the blood to the liver by way of the hepatic portal system. If not removed by hepatocytes, they enter the general circulation.

Absorption of Lipids

All dietary lipids are absorbed via simple diffusion. Adults absorb about 95% of the lipids present in the small intestine; due to their lower production of bile, newborn infants absorb only about 85% of lipids. As a result of their emulsification and digestion, triglycerides are broken down into monoglycerides and fatty acids. Recall that lingual and pancreatic lipases remove two of the three fatty acids from glycerol during digestion of a triglyceride; the other fatty acid remains attached to glycerol, thus forming a monoglyceride. The small amount of short-chain fatty acids (having

fewer than 10–12 carbon atoms) in the diet passes into the epithelial cells via simple diffusion and follows the same route taken by monosaccharides and amino acids into a blood capillary of a villus.

Most dietary fatty acids, however, are long-chain fatty acids. They and monoglycerides reach the bloodstream by a different route and require bile for adequate absorption. Bile salts are amphipathic; they have both polar and nonpolar (hydrophobic) portions. Thus, they can form tiny spheres called micelles, which are 2–10 nm in diameter and include 20–50 bile salt molecules. Because they are small and have the polar portions of bile salt molecules at their surface, micelles can dissolve in the water of intestinal fluid. In contrast, partially digested dietary lipids can dissolve in the nonpolar central core of micelles. It is in this form that fatty acids and monoglycerides reach the epithelial cells of the villi.

At the apical surface of the epithelial cells, fatty acids and monoglycerides diffuse into the cells, leaving the micelles behind in chyme. The micelles continually repeat this ferrying function. When chyme reaches the ileum, 90%–95% of the bile salts are reabsorbed and returned by the blood to the liver salt secretion by hepatocytes into bile, reabsorption by the ileum, and resecretion into bile is called the enterohepatic circulation. Insufficient bile salts, due either to obstruction of the bile ducts or removal of the gallbladder, can result in the loss of up to 40% of dietary lipids in feces due to diminished lipid absorption. Moreover, when lipids are not absorbed properly, the fat-soluble vitamins—A, D, E, and K—are not adequately absorbed.

Within the epithelial cells, many monoglycerides are further digested by lipase to glycerol and fatty acids. The fatty acids and glycerol are then recombined to form triglycerides, which aggregate into globules along with phospholipids and cholesterol and become coated with proteins. These large (about 80 nm in diameter) spherical masses are called chylomicrons. The hydrophilic protein coat keeps the chylomicrons suspended and prevents them from sticking to each other. Chylomicrons leave the epithelial cell via exocytosis. Because they are so large and bulky, chylomicrons cannot enter blood capillaries in the small intestine; instead, they enter the much leakier lacteals. From there they are transported by way of lymphatic vessels to the thoracic duct and enter the blood at the left subclavian vein.

Within 10 minutes after their absorption, about half of the chylomicrons have already been removed from the blood as they pass through blood capillaries in the liver and adipose tissue. This removal is accomplished by an enzyme in capillary endothelial cells, called lipoprotein lipase, that breaks down triglycerides in chylomicrons and other lipoproteins into fatty acids and glycerol. The fatty acids diffuse into hepatocytes and adipose cells and combine with glycerol during resynthesis of triglycerides. Two or three hours after a meal, few chylomicrons remain in the blood.

Absorption of Electrolytes

Many of the electrolytes absorbed by the small intestine come from gastrointestinal secretions, and some are part of ingested foods and liquids. Sodium ions are

actively transported out of intestinal epithelial cells by sodium-potassium pumps (Na^+/K^+ ATPase) after they have moved into epithelial cells via diffusion and secondary active transport. Thus, most of the sodium ions in gastrointestinal secretions are reclaimed and not lost in the feces. Negatively charged bicarbonate, chloride, iodide, and nitrate ions can passively follow Na^+ or be actively transported. Calcium ions also are absorbed actively in a process stimulated by calcitriol. Other electrolytes such as iron, potassium, magnesium, and phosphate ions also are absorbed via active transport mechanisms.

Absorption of Vitamins

The fat-soluble vitamins A, D, E, and K are included with ingested dietary lipids in micelles and are absorbed via simple diffusion. Most water-soluble vitamins, such as most B vitamins and vitamin C, also are absorbed via simple diffusion. Vitamin B_{12} , however, combines with intrinsic factor produced by the stomach and the combination is absorbed in the ileum via an active transport mechanism.

Absorption of Water

The total volume of fluid that enters the small intestine each day—about 9.3 L—comes from ingestion of liquids (about 2.3 L) and from various gastrointestinal secretions (about 7.0 L). The small intestine absorbs about 8.3 L of the fluid; the remainder passes into the large intestine, where most of the rest of it (about 0.9 L) is also absorbed. Total water excretion in the feces is normally limited to a tenth of a liter per day.

All water absorption in the GI tract occurs via osmosis from the lumen of the intestines through epithelial cells and into blood capillaries. Because water can move across the intestinal mucosa in both directions, the absorption of water from the small intestine depends on the absorption of electrolytes and nutrients to maintain an osmotic balance with the blood. The absorbed electrolytes, monosaccharides, and amino acids establish a concentration gradient for water that promotes water absorption via osmosis. [Table 1.2](#) summarizes the digestive activities of the pancreas, liver, gallbladder, and small intestine.

Mechanical Digestion in the Large Intestine

The passage of chyme from the ileum into the cecum is regulated by the action of the ileocecal sphincter. Normally, the valve remains partially closed so that the passage of chyme into the cecum usually occurs slowly. Immediately after a meal, a gastroileal reflex intensifies ileal peristalsis and forces any chyme in the ileum into the cecum. The hormone gastrin also relaxes the sphincter. Whenever the cecum is distended, the degree of contraction of the ileocecal sphincter intensifies.

Movements of the colon begin when substances pass the ileocecal sphincter. Because chyme moves through the small intestine at a fairly constant rate, the time

Table 1.2 Summary of Digestive Activities in the Pancreas, Liver, Gallbladder, and Small Intestine

Structure	Activity
Pancreas	Delivers pancreatic juice into the duodenum via the pancreatic duct.
Liver	Produces bile (bile salts) necessary for emulsification and absorption of lipids.
Gallbladder	Stores, concentrates, and delivers bile into the duodenum via the common bile duct.
Small Intestine	Major site of digestion and absorption of nutrients and water in the gastrointestinal tract.
Mucosa/Submucosa	
Intestinal glands	Secrete intestinal juice.
Duodenal (Brunner's) glands	Secrete alkaline fluid to buffer stomach acids, and mucus for protection and lubrication.
Microvilli	Microscopic, membrane-covered projections of epithelial cells that contain brush-border enzymes and that increase the surface area for digestion and absorption.
Villi	Fingerlike projections of mucosa that are the sites of absorption of digested food and that increase the surface area for digestion and absorption.
Circular folds	Folds of mucosa and submucosa that increase the surface area for digestion and absorption.
Muscularis	
Segmentation	Consists of alternating contractions of circular smooth muscle fibers that produce segmentation and resegmentation of sections of the small intestine; mixes chyme with digestive juices and brings food into contact with the mucosa for absorption.
Migrating motility complex (MMC)	A type of peristalsis consisting of waves of contraction and relaxation of circular and longitudinal smooth muscle fibers passing the length of the small intestine; moves chyme toward ileocecal sphincter.

required for a meal to pass into the colon is determined by gastric emptying time. As food passes through the ileocecal sphincter, it fills the cecum and accumulates in the ascending colon.

One movement characteristic of the large intestine is haustral churning. In this process, the haustra remain relaxed and become distended while they fill up. When the distension reaches a certain point, the walls contract and squeeze the contents into the next haustrum. Peristalsis also occurs, although at a slower rate (3–12 contractions per minute) than in more proximal portions of the tract. A final type of movement is mass peristalsis, a strong peristaltic wave that begins at about the middle of the transverse colon and quickly drives the contents of the colon into the rectum. Because food in the stomach initiates this gastrocolic reflex in the colon, mass peristalsis usually takes place three or four times a day, during or immediately after a meal.

Chemical Digestion in the Large Intestine

The final stage of digestion occurs in the colon through the activity of bacteria that inhabit the lumen. Mucus is secreted by the glands of the large intestine, but no

enzymes are secreted. Chyme is prepared for elimination by the action of bacteria, which ferment any remaining carbohydrates and release hydrogen, carbon dioxide, and methane gases. These gases contribute to flatus in the colon, termed flatulence when it is excessive. Bacteria also convert any remaining proteins to amino acids and break down the amino acids into simpler substances: indole, skatole, hydrogen sulfide, and fatty acids. Some of the indole and skatole is eliminated in the feces and contributes to their odor; the rest is absorbed and transported to the liver, where these compounds are converted to less toxic compounds and excreted in the urine. Bacteria also decompose bilirubin to simpler pigments, including stercobilin, which give feces their brown color. Several vitamins needed for normal metabolism, including some B vitamins and vitamin K, are bacterial products that are absorbed in the colon.

Absorption and Feces Formation in the Large Intestine

By the time chyme has remained in the large intestine 3–10 hours, it has become solid or semisolid because of water absorption and is now called feces. Chemically, feces consist of water, inorganic salts, sloughed-off epithelial cells from the mucosa of the gastrointestinal tract, bacteria, products of bacterial decomposition, unabsorbed digested materials, and indigestible parts of food.

Although 90% of all water absorption occurs in the small intestine, the large intestine absorbs enough to make it an important organ in maintaining the body's water balance. Of the 0.5–1.0 L of water that enters the large intestine, all but about 100–200 mL is absorbed via osmosis. The large intestine also absorbs ions, including sodium and chloride, and some vitamins.

Occult Blood

The term occult blood refers to blood that is hidden; it is not detectable by the human eye. The main diagnostic value of occult blood testing is to screen for colorectal cancer. Two substances often examined for occult blood are feces and urine. Several types of products are available for at-home testing for hidden blood in feces. The tests are based on color changes when reagents are added to feces. The presence of occult blood in urine may be detected at home by using dip-and-read reagent strips.

The Defecation Reflex

Mass peristaltic movements push fecal material from the sigmoid colon into the rectum. The resulting distention of the rectal wall stimulates stretch receptors, which initiates a defecation reflex that empties the rectum. The defecation reflex occurs as follows: In response to distention of the rectal wall, the receptors send sensory nerve impulses to the sacral spinal cord. Motor impulses from the cord travel along parasympathetic nerves back to the descending colon, sigmoid colon, rectum, and anus. The resulting contraction of the longitudinal rectal muscles shortens the rectum,

thereby increasing the pressure within it. This pressure, along with voluntary contractions of the diaphragm and abdominal muscles, plus parasympathetic stimulation, open the internal anal sphincter.

Diarrhea is an increase in the frequency, volume, and fluid content of the feces caused by increased motility of and decreased absorption by the intestines. When chyme passes too quickly through the small intestine and feces pass too quickly through the large intestine, there is not enough time for absorption. Frequent diarrhea can result in dehydration and electrolyte imbalances. Excessive motility may be caused by lactose intolerance, stress, and microbes that irritate the gastrointestinal mucosa.

Constipation refers to infrequent or difficult defecation caused by decreased motility of the intestines. Because the feces remain in the colon for prolonged periods, excessive water absorption occurs, and the feces become dry and hard. Constipation may be caused by poor habits (delaying defecation), spasms of the colon, insufficient fiber in the diet, inadequate fluid intake, lack of exercise, emotional stress, and certain drugs. A common treatment is a mild laxative, such as milk of magnesia, which induces defecation. However, many physicians maintain that laxatives are habit-forming, and that adding fiber to the diet, increasing the amount of exercise, and increasing fluid intake are safer ways of controlling this common problem.

Dietary Fiber

Dietary fiber consists of indigestible plant carbohydrates—such as cellulose, lignin, and pectin—found in fruits, vegetables, grains, and beans. Insoluble fiber, which does not dissolve in water, includes the woody or structural parts of plants such as the skins of fruits and vegetables and the bran coating around wheat and corn kernels. Insoluble fiber passes through the GI tract largely unchanged but speeds up the passage of material through the tract. Soluble fiber, which does dissolve in water, forms a gel that slows the passage of material through the tract. It is found in abundance in beans, oats, barley, broccoli, prunes, apples, and citrus fruits.

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CHAPTER 2

**Methods for the Analysis of
Gastrointestinal Function**

Robin C. Guy

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INTRODUCTION

This chapter will primarily discuss nonclinical methods of analyses needed to assist in the interpretation of gastrointestinal effects, in addition to discussions of models used for specific functionality assessments. Assessments of the safety pharmacology of the gastrointestinal system are found in the S7A guideline [1]. It states that:

“Effects of the test substance on the gastrointestinal system should be assessed. For example, gastric secretion, gastrointestinal injury potential, bile secretion, transit time *in vivo*, ileal contraction *in vitro*, gastric pH measurement, and pooling can be used.”

Determination of the selection of a model is not cut-and-dry. For example, for *in vitro* analyses, numerous endogenous interactions are not possible. Homogenation of tissue, for instance, usually inactivates various messenger systems, as in the adenylate cyclase systems and ions channels, as in Ca^{2+} channels. However, *in vitro* studies can provide beneficial data [1]. In addition, many *in vivo* responses are affected by a variety of factors. Responses may be different in a variety of situation, including anesthetized animals versus un-anesthetized, in surgical situations with open abdomens versus post-surgical animals, and fed versus fasted animals. For both *in vitro* or *in vivo* studies, tissues may release chemicals (e.g., prostaglandins, leukotrienes, enzymes) when tissues are activated, dissected, damaged, made ischemic, and exposed to unusual stress. As another example, myogenic activities of the gastrointestinal muscles are affected by *in vitro* conditions, as the frequency is reduced from uncoupling of higher frequency oscillators or from coupled oscillators originating from the same of different muscle layers. *In vitro* studies are also used to observe the smooth muscle, and isolated duodenum, ileum, and colon tissues are used in these studies. Any study conducted *in vitro* or *in vivo* must take a variety of factors into consideration and both qualitative and quantitative conclusions in addition to extrapolations should be drawn on a battery of studies, not just one independent study. Additional factors to consider are reviewed by Daniel et al. [2].

CLINICAL OBSERVATIONS

Observation of animals and their cages is the least obtrusive method to obtain general data on gastrointestinal function. Fecal consistency is generally observed in toxicology studies, as would be signs of emesis. Since clinical diagnoses (e.g., diarrhea, constipation, blood in feces) are not routinely incorporated into cage-side observations, other terminology is incorporated, for example, watery stool, few feces present, red material in feces, etc.

TRANSIT

Transit defines how materials mix and progress through the gastrointestinal tract and is one of the main functions of the gastrointestinal tract. Different areas of the gastrointestinal tract have distinct functions. Transit is controlled primarily by three mechanisms, myogenic, neural and chemical [2]. Myogenic refers to electrical control activity (ECA) generated in the smooth muscle and affects the resting membrane potential, frequency and periodic oscillations of membrane potential, and phase relationships in adjacent cells. Neural refers to the control of the extrinsic and intrinsic nerves. Chemical refers to the various substances released from the nerves and the cells and glands. Transit occurs in the esophagus, stomach, small intestines, and large intestines. Gastrointestinal motility may be studied in isolated single smooth muscle cell preparations as these are widely available in a variety of species including guinea pig, dog, rabbit, and human [1]. Cell lines for the *in vitro* analyses of gastrointestinal function and analyses are described in Zweibaum et al. [33]. In addition, human intestinal smooth muscle cells have been shown to retain their sensitivity to stimuli for at least 6 days into culture.

Esophageal Transit

The motility of the esophagus is relatively easy to study in man, since it is readily accessible. Control of motility is more difficult to analyze, since normal human tissue is not readily available. The human esophagus consists of striated muscle in the proximal one-third to one-half, and smooth muscle in the remaining length [3]. In common laboratory animals, namely the dog, cat, rat and mouse, the esophagus is primarily composed of striated muscle. Many marsupials and some non-human primates have tissue in similar proportions as man. The use of an appropriate laboratory animal model maybe restrictive, but the North American opossum (*Didelphys* sp.) has been used as an animal model [3]. Additional animals used to study esophageal function have included the Australian possum and non-human primates.

The movement of food in the esophagus has been shown to be inhibited by muscarinic blockers (antimuscarinic, anticholinergic) [4].

An esophagoscopy is a convenient method for the observation of the esophagus, especially the lower portion of the esophagus [5]. It is also useful for obtaining biopsy samples and cytologic brushings, in the case of suspected cancer. The animal may need to be anesthetized to prevent discomfort through the procedure.

Intraluminal pressure studies of the esophagus are used to detect achalasia and motor disorders of the muscle tissue and sphincter. A thin catheter, with pressure sensors imbedded in the wall, is inserted into the esophagus. The outer end of the catheter is attached to a transducer that records pressure. The catheter records the duration and sequence of esophageal contractions, including assessments of the integrated muscle activities of the different types of muscle in the esophageal wall.

In addition, *in vivo* studies have been conducted where pressure gauges have been surgically implanted at specific points along the esophagus to record findings [6].

Verapamil is a calcium channel blocker that was tested to determine its efficacy as a treatment for esophageal disorders [7]. In the baboon, verapamil inhibits the amplitude of esophageal contractions in the smooth muscle section of the esophagus and decreases the lower esophageal sphincter pressure. To determine this, baboons were fasted for 24 hours, and then anesthetized with ketamine hydrochloride prior to placement in a restraint chair. An esophageal manometric catheter was inserted through the nose and into the stomach. The catheter was slowly pulled across the gastroesophageal junction until the distal sleeve sensor recorded lower esophageal sphincter pressure. Based on the design of the catheter, this placement resulted in the measurement of primary striated muscle activity and smooth muscle activity.

The rhesus monkey (*Macaca mulatta*) was used in manometric studies to compare data obtained from similar human studies [8]. In this study, a small triple-lumen catheter was constructed. Animals were fasted overnight and placed supine in a restraining box. A pneumographic belt was placed around the chest or upper abdomen to record breathing. A disruption in the rhythmic respiratory waves indicated the act of swallowing. The catheter was placed through the mouth to the stomach. The catheter was then withdrawn at 0.5 to 1 cm increments to record pressure at each site. The study measured resting gastric pressure, gastroesophageal pressure gradient, the point of respiratory phase reversal, sphincter tone (inferior and superior esophageal sphincter), primary and secondary peristalsis (at the upper, middle, and lower third of the esophagus), and diffuse spasms. This study concluded that there were functional esophageal similarities in the macaque and human.

Myoelectric activity of the esophagus can also be recorded *in vivo* utilizing bipolar or monopolar electrodes [6]. This provides a more precise measurement of muscle activity than does the pressure recording. These electrodes are sewn into the outside of the esophagus. The measurements are an indication of the sum of the activities in the various muscle layers.

Motor activity can also be studied *in vitro* [6]. Strips of the esophageal muscle tissue are bathed in oxygenated physiologic solutions. These tissues are connected to force transducers. Chemicals of interest may be added to the solutions to determine their effect on motor function. In addition, intracellular microelectrodes may be used to determine the electrical activity of individual esophageal smooth muscle cells [6].

Forceful reverse peristalsis occurs during emesis. Emesis can occur in the dog and cat and most of the time in monkeys, but not in the rat, mouse or rabbit. Prolonged or repeated emesis may be responsible for alterations in normal electrolyte concentrations. Blood samples may be obtained to determine if electrolytes have been affected.

Gastric Emptying

Gastric emptying is the process which the contents of the stomach, chyme, are moved into the duodenum. Three types of movements occur in the full stomach:

peristaltic waves, systolic contractions of the terminal antrum, and reduction of the size of the stomach. It has been determined that liquids leave the stomach earlier than solids, and smaller solid particles leave the stomach earlier than larger particles [9,10].

At fasting, the intraluminal pressure in the stomach is equal to the intra-abdominal pressure [9]. In a study to determine the effects of pressure on a full stomach, pressure was recorded in a vagally innervated pouch of the fundus of the stomach of a dog.

The effects of gastric emptying may be measured by the x-ray of radio-opaque particles [11]. Beagle dogs were fasted for approximately 15 hours. Various sized radio-opaque particles (1–3 mm in diameter) were prepared and placed into size 000 gelatin capsules. The capsules were administered orally to the dogs either fasted or at 5 minutes after a meal. Following administration of the capsule, the dogs were x-rayed every 2 to 5 minutes for the first 15 minutes, and every 15 minutes thereafter to determine the location of the particles.

An assessment of the difference in the motility of large and small particles, as well as liquids in the canine stomach, was described [10]. A duodenal fistula was surgically implanted in the dogs. Cubes of beef liver were labeled with $^{57}\text{(Co)}$ cyanocobalamine and were fed to the dogs as the digestible solid food. Plastic spheres (7 mm) were fed as indigestible solid food. The liquid food was a dextrose solution tagged with polyethylene [1,2- ^3H]glycol. Samples of the duodenal contents were aspirated through a tube inserted into the fistula at various time points. The radioactivity for each marker was determined. The effects of test material administered prior to feeding on the motility of different food particle size and consistency can be determined.

The effect of liquid nutrient loads on gastric emptying was assessed in the rhesus monkey, *Macaca mulatta* [12]. Intragastric cannulas (41 mm ID $\times$ 76 mm OD) were surgically implanted in the male monkeys in the stomach dorsal to the greater curvature. The end of the cannula was externalized by the left side of the spine and through the intercostals space at the level of the stomach. Canvas jackets were placed on the monkeys for protection. In addition, the exterior cannula was placed in a flexible steel cable that extended to the back of the cage so that infusions could be performed without handling the monkey. The monkeys' movements were unrestricted. Monkeys were allowed to recover from surgery for 2 weeks before the gastric emptying experiments. Prior to the experiments, animals were fasted for 16 hours. In addition, to determine the status of the contents in the stomach 1 hour prior to the start of the study, 50 mL of isotonic saline (0.15 M NaCl) at 37 degrees Celsius was infused, then immediately withdrawn from the stomach. If the stomach was empty of any food contents, the studies were conducted. Test materials were administered to the monkeys followed by the liquid nutrient loading (100 mL) 30 minutes later. A marker consisting of nonnutritive phenol red dye was mixed with the test loads. Ten minutes later, the material remaining in the stomach was withdrawn and the stomach was repeatedly washed with saline until traces of the dye were no longer present. The volume of material remaining in the stomach was then determined by dye dilution spectrophotometry. This method may be used to determine the gastric inhibitory effect of a test material.

Other factors can affect transit time. Liquid gastric emptying time was delayed in diabetic hyperglycemic rats [13]. Intraperitoneal injections of a synthetic cholecystokinin slowed the gastric emptying of both liquid and solid food in the rat [14]. In addition, electromechanical changes from canine fundal and antral smooth muscle preparations have been shown to have different responses to nerve stimulation which accounts for their physiologic responses *in vivo* [15].

Motility of the Small Intestine

The chyme that leaves the stomach is moved down into the duodenum. Once in the intestine, it moves slowly via short, weak propulsive movements to allow for digestion and absorption. In the dog, propulsive activity has been measured by injecting body-temperature isotonic saline into the upper small intestine at a pressure of 1 Hg [9]. The amount of time it takes for a specific volume of fluid to be transported is an indication of the intestinal motility. The rate of transport is affected by feeding, as the rate is reduced by 80%. This reduction is due to increased intestinal contractions, which narrows the lumen, thereby increasing resistance to transport. The rate of transport is also affected by atonic conditions, where movement would be rapid since there is less resistance. Morphine and castor oil have also been shown to effect transit, and these could be used as positive controls. Morphine causes inhibitory neurons to be suppressed, thereby causing spasms in the duodenum. Morphine also increases phasic and tonic activities of the smooth muscles in the gastrointestinal tract. These effects slow down transport and may cause increased absorption of water from the feces, which may lead to constipation. Castor oil (ricinoleic acid) causes a transient increased activity followed by a prolonged inhibition [9].

Safety pharmacology studies have been designed to determine the effects of drugs on the gastrointestinal tract. [Chapter 3](#) is devoted to this discussion. However, one study determines the gastrointestinal propulsion effects of a drug and will be discussed briefly. In this study, the test article is administered to a rat or mouse (5/sex/group). A 5% or 10% suspension of activated charcoal in aqueous methylcellulose is administered by oral gavage to the animal. Rodents are then euthanized 30 minutes after receiving the charcoal suspension. The intestines are removed and laid out on moist paper. The distance that the charcoal traveled as a percent of the overall length of the intestine is evaluated and compared to control, vehicle control and any other dose group animals to determine increased or decreased transit.

Rats were used to examine small intestinal transit specifically [16]. Since gastric emptying is variable, intraduodenal injections were made for consistency. Male rats weighing approximately 200 to 300 kg were used. Rats underwent intraduodenal intubation 5 days prior to the study. Further details are described in detail in the manuscript. The procedure did not obstruct the duodenum or interfere with normal eating and drinking. A variety of treatments were used to alter intestinal propulsion. Drugs (0.5 mL) were administered 2 hours prior to killing the animals. The solution administered intraduodenally was Krebs 0.1 M phosphate buffer with 100 mg glucose, 2.0 mg polyethylene glycol, 0.5 mg phenol red, and 100 μ C of chromium as the sodium salt of chromate ($\text{Na}_2^{51}\text{CrO}_4$). Control animals were killed at 0, 15, 30, and

60 minutes after the intraduodenal injections. To prevent the movement of ^{51}Cr during dissection and removal of the intestines, ligatures were placed at various points as soon as the animals were killed. In increasing magnitude of slowed propulsion, morphine sulfate (10 mg/kg intramuscularly with epinephrine), chlorisondamine chloride (ganglionic blocking agent, 8 mg/kg subcutaneously), and mecamlamine hydrochloride (ganglionic blocking agent, 10 mg/kg subcutaneously) had a greater retarding effect on propulsion than other drugs. The leading edge traveled a shorter distance in the intestine than in the control animals. Accelerated propulsion was obtained by irradiation (whole body radiation, 1400 R at 75 R/minute).

Another study determined the effects of certain drugs on the motility of the rat small intestine and investigated whether motility affects the small intestinal bacterial flora [17]. Under conditions of extreme stagnation of motility, significant bacterial overgrowth was found to occur.

To determine effects on the myoelectric complex of the movement of the fasting intestine, unanaesthetized dogs may be used [9]. Dogs are fasted for 12 hours before observations are performed. The dogs previously had surgically implanted electrodes placed in a row on the serosal surface of the gastric antrum and along the entire length of the small intestine. The leads from the electrodes are fed through to the surface of the body. Strain gauges may be sewn into muscle to determine movement. Catheters may be inserted into the lumen to measure pressure. After the animal has recovered from surgery, the electrical activity can then be monitored under a variety of test conditions. Contrast medium can also be injected through the catheter so that cinefluorography can be utilized to observe the motility of the intestinal contents.

Motility of the Large Intestine

Many of the preceding techniques can be used to track transit in the large intestine. However, the motility of the large intestines is not as regular and is more variable than the stomach and small intestines. As with the small intestine and the stomach, specific hormones and peptides may affect the motility in the large intestines. Stimulation of motility is produced by gastrin, cholecystokinin, substance P, and enkephalins, while motility is inhibited by glucagons, vasoactive intestinal polypeptide, and secretin [4]. A comparative anatomical study of the distal end of the large intestine was conducted in eight mammalian species [18]. Due to the extreme differences in the anatomy and physiology of the large intestine, there appears to be no ideal animal model for the large intestines [19]. However, specific aspects of large intestinal motility have been investigated.

The motility in the large intestines was examined in the cat [20]. Highly sensitive semiconductor strain gage transducers were implanted extraluminally to detect large intestinal motility as it is affected by stimulation of areas of the central nervous system. Three transducers per animal were attached to the surface of the colon transversely. These were attached near the cecum, at the middle part of the colon and at the sigmoid colon. The transducers did not appear to interfere with the spontaneous activity of the colon. Intraluminal transducers were also surgically placed in the

colon through small antimesenteric incisions of the intestinal wall. Pressure was recorded by the intraluminal transducers by means of balloons filled with water, or by open-ended tubes filled with saline. The intraluminal balloons were sometimes observed to induce some degree of colonic activity. In addition, solid or semisolid fecal mass blocked the open-ended tubing on occasion. It was determined that for the feline colon, the extraluminal strain gage transducer was able to record the movements of various segments of the colon directly, while intraluminal recordings both with balloons or open-ended tubes were only able to record activity in a specific area. Although the extraluminal transducer provided more sensitive readings, it was not able to differentiate between contractile activity in longitudinal and circular muscle layers. In the same study, it was determined that deep anesthesia was associated with decreased peristaltic motility, and minimal to no effects of nerve stimulation. However, light anesthesia produced artifacts and a muscle relaxing agent was needed.

In addition, many drugs have effects on the function of the large intestine [19,21]. These may be used to analyze or compare the motility of the colon or a colonic effect in *in vivo* or *in vitro* studies. The following list should not be construed as complete or for drugs that affect the entire large intestine, as that may not be the case with all of the drugs. Alpha-adrenergic drugs increase contractions in circular muscle but inhibit contractions on the longitudinal muscle of the cat colon. Beta-agonists and dopamine cause inhibition of motility. The effects of atropine and other anticholinergics on motility vary depending on the methodology; however, there may be a very small and brief inhibitory effect of atropine *in vivo*. *In vitro* studies with atropine showed no effect on the electromyogram or spontaneous contractions of the circular layer of the cat colon; however, it decreased spontaneous contractions of the longitudinal muscle layer. Substance P increases potent contractions of the circular muscle layer. Met-enkephalin increases motility in the cat intestine. Bradykinin inhibits motility in both layers of the muscle *in vivo*, but at high concentrations *in vitro*, stimulates the longitudinal muscle layer. Cholecystokinin stimulates the circular muscle layers *in vivo*. Angiotensin increases the contractions in both layers *in vitro*. Morphine increases the intensity of the spike activity and prolongs the duration of slow waves in the cat colon *in vitro*. Gamma-aminobutyric acid induces a temporary relaxation in both layers of the guinea pig colon. As discussed for the small intestine, castor oil (ricinoleic acid) increases colonic activity.

Numerous cell lines for colonic function assays have been established and are described in Zweibaum et al. [33].

SECRETION

Secretions in the gastrointestinal tract include gastric acid, hormones, bile, enzymes electrolytes, and mucus. Some examples of tests for the analyses of these secretions are listed below, though this is not a complete list.

Gastric acid determination is a procedure to evaluate gastric secretion function by measuring the amount of acid secreted from the stomach [22]. This is the most

widely practiced procedure in humans [23]. In humans, it is often done in conjunction with the gastric acid stimulation test, a procedure that measures gastric acid output after injection of a drug to stimulate gastric acid secretion. In fasted animals, a flexible tube is inserted through the mouth or nasogastrically to the stomach, with proper positioning confirmed by fluoroscopy or x-ray. After a short acclimation period, specimens are obtained every 15 minutes for a period of 90 minutes. The first two specimens are discarded to eliminate gastric contents that might be affected by the stress of the intubation process. The specimens are then analyzed for gastric acid. A gastric acid stimulation test may be conducted immediately after the last gastric sampling. Pentagastrin, or a similar drug that stimulates gastric acid output, is injected subcutaneously. Specimens are collected every 15 minutes for 1 hour and analyzed for gastric secretions.

The effects of intragastric acid and pH changes may be monitored by a disposable radiotelemetric pH sensor, the Heidelberg capsule [11,24,25]. Capsules were calibrated prior to the surgery and the calibration was checked after the completion of the study. In addition, gastric juices were aspirated through a stomach tube inserted orally and the accuracy of the pH readings from the capsule was assessed by measuring the pH with a conventional pH meter. In one study, a length of surgical suture was attached to the Heidelberg capsule and held in place to ensure that the capsule would remain in the stomach [8]. In this manner, the pH of the stomach could be monitored continuously throughout the study. In another study, the capsule was allowed to traverse through the gastrointestinal tract for collection of pH data both in the stomach and the small intestine [25].

The effects of gastrointestinal hormones on function of the gastrointestinal tract have been examined. Gastric inhibitory peptide has an insulintropic action which was studied in humans, dogs, and rats [26]. Gastric inhibitory peptide is normally released after ingestion of carbohydrates and fats and after intraduodenal infusions of amino acids. Dogs with indwelling venous catheters and gastric fistulas were utilized and were fed a peptone meal to determine the response of gastric inhibitory peptide and serum insulin and glucose levels. This study was able to detect gastric inhibitory peptide release due to the utilization of a sensitive radioimmunoassay method for measuring gastric inhibitory peptide. A gastrin-deficient mouse was genetically developed to investigate the role of this peptide hormone in the development and function of the stomach [27]. It was determined that gastrin is critical for the function of the acid secretory system.

Bile is secreted from the liver. It is stored in man and some other animals in the gall bladder and released into the duodenum. If the functionality of the liver, gall bladder or ducts is compromised, changes in blood chemistry values may be observed. Extrahepatic cholestasis occurs when the flow of bile is obstructed and bile acids leak into the plasma [28]. Other changes may be observed in bilirubin, enzymes, and electrolytes.

Testing for pancreatic exocrine function can be conducted in a variety of assays [23]. These include tests that quantitate pancreatic secretion through gastroduodenal intubation, studies that assess exocrine function indirectly using an orally administered test substance, tests that measure fat or pancreatic enzyme activity in feces, and

measurement of pancreatic serum enzyme activity. Pancreatic secretions contain an alkaline fluid to help neutralize the acid entering the duodenum, and enzymes. In humans, cats, and dogs, the pancreas secretes primarily during digestion [29]. Rats, sheep, and rabbits are continuous feeders and their pancreas secretes continuously. The centroacinar cells and duct cells secrete the alkaline fluid, and secretion of this fluid is stimulated by secretin and cholecystokinin [23,29–31]. Any chemical that affects the function of these cells will affect the material in the gastrointestinal tract. Pancreatic fluid may be collected for analysis from animals with a surgically placed duodenal fistula with a tube placed opposite of the pancreatic duct. In addition, secretions of amylase can be measured from pancreatic lobule preparations from which the lobules were excised and incubated in a medium [32].

Calcium is secreted primarily in the jejunum and ileum and is stimulated by mucosal sodium and somatostatin [33].

Rat cell lines have been established. The effects of metabolic function of the IEC cell line from rat small intestines have been determined in *in vitro* studies [34]. The RCC rat colonic cell line was obtained from tumors induced chemically [34]. The RCC-5 cell line has lost its ability to produce mucus *in vitro*. Therefore, it is used for the study of hormonal, nutritional and physical conditions necessary for mucus differentiation of colon cells.

GALL BLADDER, BILE DUCTS, AND PANCREAS

Myoelectric activity is important in the proper function of the gastrointestinal tract. Gall bladder filling and emptying and duodenal bile acid delivery during fasting are cyclically coordinated with myoelectric activity [35]. To determine this, dogs were surgically implanted with a duodenal cannula and eight bipolar electrodes that were placed from the duodenum to the terminal ileum. During continuous intravenous infusion of [^{14}C]taurocholic acid, fasting myoelectric activity and duodenal delivery of the taurocholic acid were recorded under different conditions. Therefore, monitoring these parameters may help expose functional changes related to gall bladder filling and emptying in addition to duodenal bile acid distribution.

A study was conducted to determine the effects of sphincter of Oddi obstruction due to topical administration of carbachol on the sphincter [36]. This study assayed functionality through measurements of sphincter of Oddi motility, trans-sphincter flow, pancreatic duct pressure, pancreatic exocrine secretion, plasma amylase levels and pancreatic tissue damage. The Australian Brush Tailed possum (*Trichosurus vulpecula*) was used due to its similarity to the human pancreatic and bile ducts.

The effects of bile duct cannulation on functional gastrointestinal transit time were studied in F-344-rats [37]. Rats were subjected to surgery consisting of laparotomy with gut manipulation, sham bile duct cannulation, actual bile duct cannulation, or halothane anesthesia alone. Rats received radio-opaque barium-sulfate orally. Radiographic images were made within 2 minutes and periodically to locate movement of the barium through the gastrointestinal tract. It was determined that bile duct cannulation markedly slowed gastrointestinal motility.

ABSORPTION

Selection of an animal model of gastrointestinal absorption depends upon the specific function to be studied [38]. Differences in gastrointestinal absorption of various species are reviewed based on function, anatomical variation, gastrointestinal tract pH, flora, functional differences, absorption of inorganics and organics, and confounding variables. In a study of 38 organic compounds, more than a third appeared to be differently absorbed by animal models as compared to human subjects [38].

Absorption can be affected by a variety of parameters [39]. When selecting a study design to assess absorption-related features of the gastrointestinal tract certain parameters need to be taken into consideration. The following parameters are likely to have a major effect on the kinetics of gastrointestinal absorption: vascularity, transit time, surface area, contents, enterohepatic cycling, and the chemical properties of the compound studied.

Fat malabsorption can be measured from fat in fecal samples [23,40,41]. Stool samples are pooled, mixed with water and homogenized. A sample may then be analyzed by hydrolysis, extraction, and titration of the fatty acids. Steatorrhea is a condition where more fat in the stool is present than normal, an indication of decreased fat absorption. A coagulation test for the determination of prothrombin time is an indication of Vitamin K absorption [39]. An increase in prothrombin time may also be due to steatorrhea. Fat malabsorption has also been determined in breath tests to detect $^{14}\text{CO}_2$ levels after ingestion of meals containing ^{14}C labeled fats or triglycerides [42–44] or in nonradioactive breath tests [45].

One indication of altered carbohydrate absorption is in an oral glucose tolerance test. A fasting blood sample is obtained, followed by samples every 30 minutes for approximately 2 hours after oral administration of a known amount of glucose dissolved in water. High values may indicate diabetes mellitus. Breath tests are also available to evaluate carbohydrate malabsorption by evaluating H_2 after the administration of carbohydrates [23]. Lactose intolerance testing may also be utilized [23].

Protein absorption analyses of fecal samples are not normally conducted, as amino acids are partially catabolized and reused for protein synthesis [40] and are metabolized by bacterial in the large intestine.

Iron may become malabsorption if the duodenum is damaged, as iron normally is absorbed in this location [39,40]. A blood smear from an animal with deficient iron absorption shows hypochromia and microcytosis. Serum iron levels would be low with malabsorption. Additional assays for absorption of iron is whole body counts and fecal recovery. In these studies, after an overnight fast, animals are given oral dosages of 10 mcgCi ^{59}Fe . A whole-body count is made 4 hours later. For fecal recovery, feces are collected and radioactivity counted until less than 1% of the administered dose appears.

Absorption may be measured *in situ*, as described in a study on cadmium [46]. Under surgical procedures, a loop of the small intestine was isolated and cadmium was injected intraluminally. The mesenteric venous (portal) blood coming from the loop was collected for 90 minutes and was analyzed for cadmium.

MICROBIAL FLORA

Normally, the lumen of the gastrointestinal tract contains many micro-organisms that assist with typical gastrointestinal functionality. The lumen is protected against direct exposure of the micro-organisms by a thick mucus layer. An imbalance of the micro-organisms may be due to irritation, an infection or xenobiotics, and could cause the gastrointestinal tract to be damaged, or have impaired activity.

Imazalil has been observed to alter the composition of the gut microbiota after 15-week chronic exposure [47].

Escherichia coli enterotoxin-induced diarrhea has been shown to produce changes in intestinal absorption and permeability [48,49]. A simple rectal culture test can identify and isolate organisms. A cotton swab is gently inserted into the rectum, rotated, and withdrawn. A smear is placed on culture media and incubated. The culture is observed at regular intervals. Organisms can then be identified and isolated.

The small intestine may also be sampled for bacterial flora. One method is a needle aspiration of the intestines. Another method consists utilizes a double-lumen radio-opaque tube [50]. The tube is placed down the esophagus of fasted animals and positioned in the mid-jejunum area. Aspirates are taken and the tube is withdrawn. Samples are then plated for both aerobic and anaerobic cultures.

Many intestinal bacteria are able to deconjugate bile acids [51]. This normally occurs in the large intestine; however, if the deconjugation takes place in the small intestine, absorption of [^{14}C]glycine occurs. The glycine, administered orally as ^{14}C -glycine-glycocholic acid, is completely metabolized and produces $^{14}\text{CO}_2$, which may be measured in the breath.

Urinalyses may also be performed to detect an excessive growth of bacteria in the small intestines [51]. Analysis for the presence of indican (indoxyl sulfate) may be conducted. Indoles are produced by bacteria, particularly *Escherichia coli* and *Bacteroides*.

IMMUNE FUNCTION

The gastrointestinal tract has ample immune function activity [51,52]. Alteration of immune modulating cells and gut tissues are linked to abnormal function of the gastrointestinal tract. Mucosal tissues have glandular secretions that secreted on the epithelial surface. Most antibody activity in mucosal secretions is of secretory IgA, but other factors need to be taken into consideration during a local immune response, including cellular and humoral mechanisms. There are many types of mucosal tissues, including gut-associated lymphoid tissue (Peyer's patches and isolated lymphoid follicles) and bronchus-associated lymphoid tissue. Other antibody activity and cells include, but are not limited to, IgG, IgM, T-lymphocytes, natural killer cells, mast cells, macrophages, goblet cells, and precursor B-cells from Peyer's patches.

Changes in the number of mast cells have been observed in a variety of immune responses in different tissues, including the gastrointestinal tract [53]. Mast cells have been found to influence the immunologic, physiologic, and pathologic functions of the gastrointestinal tract, including normal functions of the stomach and intestine. Mast cell-deficient mice models have been identified (WBB6F1-W/W^v and WCB6F1-SI/SI^d). These animals may be used in studies to assess numbers, phenotypic characteristics, and anatomical distribution of mast cells potentially participating in responses, including mast cell degranulation.

Normal immune function of the intestines is absent in inflammatory bowel diseases, including Crohn's disease. There are many models of inflammatory bowel disease, but less progress has been made for models for fibrotic lesions [54]. A concern is that it is difficult to analyze for fibrosis *in vivo*, as it is difficult to determine the difference between indirect effects caused by altered inflammation from direct effects. Both CD-1 and BALB/c mice were sedated weekly and intrarectal injections of trinitrobenzene sulfonic acid were administered [55]. Only a fraction of the animals developed severe fibrosis, and not all of those animals had persistent fibrosis after the intrarectal injections ceased.

There is a possible link between inflammatory mediators, particularly platelet activating factor, play an important role in the pathogenesis of necrotizing enterocolitis, a life-threatening gastrointestinal problem in neonates [56]. A neonatal piglet model for this inflammatory disorder was developed and the model was used to investigate the role of platelet activating factor in its pathogenesis. Rodent models were also investigated; however, the neonatal piglet model is more relevant to humans due to more similar anatomy and physiology. The piglets were anesthetized and a cuff-type electromagnetic flow probe was surgically placed around a branch of the cranial (superior) mesenteric artery. The location was selected to monitor the mesenteric blood flow for additional data to support the model. The animals were allowed to recover and the procedure for the development of the model occurred after stabilization of the readings from the monitor. The animals received an intravenous injection of an endotoxin (lipopolysaccharide, 2 mg/kg). These animals were then allowed to breathe normal room air for 45 minutes, followed by a 45-minute period of breathing 10% oxygen, then another 45-minute period of breathing room air. The animals were then killed by a lethal dose of pentobarbitone sodium and the animal was examined for gross and microscopic changes. This treatment caused a reduction of mesenteric artery blood flow in addition to the intestinal lesions that are consistent with necrotizing enterocolitis.

A change in immune functionality of the gastrointestinal system may occur due to allergy [57]. A rat model was developed to assist the study of gastrointestinal allergy. Allergic sensitivity was induced in rats to certain proteins by injecting proteins and adjuvants in various regimes. Sensitivity was also established in suckling rats by oral administration of the protein. A hypersensitive gastrointestinal reaction to challenge was demonstrated by electron microscopy, by light microscopy with the aid of conventional staining techniques, and also by a radionuclide procedure utilizing ⁵¹Cr-labeled albumin.

GASTROINTESTINAL ENDOSCOPY

Standard endoscopy scopes and cameras have been used for decades to observe the intestinal lumen. In 2000, an article was published, a Brief Communication article in *Nature*, about a wireless capsule endoscopy system that was developed [58]. Another article followed up on this wireless capsule endoscopy a few years later [59]. Types of non-digestible capsule with a camera are currently used in animal models to observe the GI tract as it proceeds through the body.

Bleeding from the gastrointestinal tract may indicate a variety of functional or pathologic problems. Bleeding and ulcers may be detected by endoscopy, but bleeding may also be detected by the presence of blood in feces and is in most cases easier than finding the bleeding site. Occult blood tests may be performed on fecal samples [60]. There are a variety of methods used to detect occult bleeding, including the use of chemical tests, microscopic examination of feces for erythrocytes or hemoglobin crystals or its derivatives, spectroscopic analyses for hemoglobin or its derivatives, and ^{51}Cr -labeled erythrocytes. Fecal occult tests may produce false positives with dark feces, which may be due to injected iron; in this case, simple testing is needed to rule out iron [61].

CLINICAL CHEMISTRY ANALYSES

Routine blood sampling and analyses are ways to get an insight into functional problems with the gastrointestinal tract [62]. For example, electrolyte and fluid imbalance may be due to emesis, diarrhea, or a variety of mechanisms for maintaining electrolyte and fluid homeostasis. Chloride secretion from the pancreas varies inversely to the bicarbonate concentration, which varies directly with flow rate. Hypocalcemia may also accompany severe pancreatic toxicity.

Glucose homeostasis is controlled by the liver and changes in that organ may produce imbalances. Hormones and other chemicals also have additional control of glucose levels, and include insulin (which is highly variable), glucagons, growth hormone, adrenaline, and cortisol. Urinary levels of glucose may also be determined to detect functional imbalances of glucose metabolism. Electrolyte metabolism may be altered during carbohydrate metabolism disturbances.

The measurement of enzymes is important for assessing gastrointestinal function, but many tests are specialized and are not common, especially in standard toxicology tests. Plasma P amylase is a specific assay for pancreatitis. Lipases are secreted by the pancreas. Alkaline phosphatase can change with gastrointestinal conditions, such as intestinal obstruction or infarction, parasitic infection, obstruction of the biliary system, contraction of the sphincter of Oddi by a specific drug, withdrawal of food and age-related changes. Alkaline phosphatase is also secreted by the bone, liver, and placenta. As the liver can affect gastrointestinal functionality, liver function testing should also be performed [63].

PAIN

Pain due to gastrointestinal disorders may be from numerous sources, including esophagitis, gastro-esophageal reflux, restriction of the esophagus, tumors, infections and inflammation, constipation, irritable bowel syndrome, gall stones, pancreatitis, and metabolic and hormone disorders. Determination of the degree and location of pain in laboratory animals is difficult to reproduce due to a communication barrier. To determine if a specific chemical causes pain in the gastrointestinal tract, one might look for gross (as a result of an endoscopy or at necropsy) or histopathological effects only as an indication of possible pain.

pH AND PRESSURE

Additional measurements may be performed to assess gastrointestinal function, including both intraluminal pH and pressure. A wireless motility capsule can be used to measure these parameters, and had been especially successful in the dog [64]. The capsule is ingested and as it proceeds down the gastrointestinal tract, it transmits pressure, temperature, and pH to an external receiver. These data may then be downloaded to a computer for analysis. A radiotelemetry Heidelberg capsule has also been used in the same manner for measurement of pH [65,66].

NAUSEA AND VOMITING

Many chemotherapeutic drugs for the treatment of cancer in addition to other drugs can cause nausea and vomiting. Nausea is a subjective human sensation, but it was possible to predict between nausogenic and non-nausogenic drugs based on their preclinical gastrointestinal observations [67]. From the article, the most common preclinical gastrointestinal effects observed in drugs that are associated with nausea were vomiting, salivary hypersecretion and constipation. In addition, combination of common preclinical effects (e.g., vomiting, diarrhea, salivary hypersecretion) increased probability of a drug-induced nausea in humans.

Investigations into these effects are performed in species that do exhibit emesis; the dog, pig and ferret [68,69] are examples. Rats do not exhibit emesis, and therefore are not used for these types of studies.

Additional methods are used in studies to determine early anti-emetic activity in addition to early and delayed emesis through telemetric techniques.

IMAGING

There are a number of imaging techniques currently used including x-rays, PET, CAT scans, and MRIs. Many contract research organizations have invested in these types of equipment, which are helpful to determine effects in non-anesthetized and

restrained animals. Contract agents may be administered to the animals to assist with the determination of multiple endpoints. A sequence of events may even be developed with some of these methods. Timing of the imaging is a consideration for the most accurate results.

HISTOLOGY

The microscopic examination of biopsy samples and tissues taken from animals on toxicology studies leads to an incredible wealth of knowledge with regard to methods for diagnosing clinical signs and other possibly unseen issues from the gastrointestinal tract. Microscopic examination results are any histopathologic changes that are made to the tissue or cell. Microscopic examinations also can clarify what was observed previously in the laboratory during the antemortem part of the study, or can explain findings observed during necropsy, or postmortem. They can also assist with the determination of toxic or adverse effects.

It is therefore critical to assure that adequate samples are taken and processed to slides which would help to provide the pathologist with meaningful and readable slides for analysis.

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CHAPTER 3

Safety Pharmacology and the GI Tract

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INTRODUCTION

Long before safety pharmacology became a familiar term, pharmacologists and toxicologists evaluated developmental compounds for adverse effects on multiple organ systems in animal models. They did this mainly to evaluate compounds early in the development process that had a spectrum of unwanted effects at doses equal to or greater than those showing “efficacy.” These studies were felt to be important in obtaining as much information as possible about, not only serious toxicological potential, but also about those properties that produced unwanted, and sometimes annoying, effects that seriously impacted on patient compliance. This is especially

true with drugs for chronic therapy where even minor side effects can affect compliance. These studies have been termed by various names: ancillary, general, developmental pharmacology, and may have been done in either discovery or development departments in pharmaceutical companies. It is only within the last 20 years that there have been guidelines to attempt to codify the number and types of studies required for regulatory approval. Although many lists of “safety pharmacology” studies have been put forth, these are just suggestions. Other than ongoing improvement of testing or effects on heart rhythm, few changes have been made in testing protocols for other areas of interest.

DEFINITION OF SAFETY PHARMACOLOGY

The origins of safety pharmacology are grounded upon observations that organ functions (like organ structures) can be toxicological targets in humans exposed to novel therapeutic agents, and that drug effects on organ functions (unlike organ structures) are not readily detected by standard toxicological testing [1]. Pre-clinical safety assessment programs are usually designed to meet the needs of early clinical studies and the International Conference on Harmonization (ICH) guidelines on non-clinical safety [2] (ICH M3 1997) or local regulatory agencies. In addition to the repeated dose toxicity studies in one rodent and one non-rodent species, genotoxicity, and safety pharmacology evaluations are critical to the full evaluation of a drug’s safety and usually are conducted prior to the start of Phase I clinical studies. Due to their relevance for human safety, the conduct of preclinical safety and pharmacokinetic studies before the start of clinical studies and granting of marketing authorization has become a critical, mandatory and fairly standardized field regulated by national and international health authorities [3]. However, there is no consistent official definition of the term “Safety Pharmacology.” Kinter et al. [4] define it as the systematic approach of investigating a candidate drug for activity to stimulate, potentiate or inhibit the activity of physiological or pharmacological responses or interaction with another drug, whether *in vitro* or *in vivo*. Safety pharmacology studies are designed to assess the potential adverse effects of a compound on the physiologic function of one or more organs or organ-systems, in either intact or acutely prepared models that are of proven relevance to humans, whether healthy or sick.

Safety pharmacology must cover pharmacologic properties at dose levels that are relevant to clinical use and not excessive. In addition, it should focus on effects that are difficult to detect within toxicity studies. Definition of safety margins may also be important and it may be important to assess the “therapeutic index.”

Safety Pharmacology Core Battery

These guidelines include a set of tests (core battery) plus other tests that may or may not be relevant or required the latest, which is PHARMACOLOGY STUDIES (2) 2010 Guidance for Industry M3(R2), Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals.

The core battery of safety pharmacology studies includes the assessment of effects on cardiovascular, central nervous, and respiratory systems, and should generally be conducted before human exposure. When warranted, supplemental and follow-up safety pharmacology studies can be conducted during later clinical development. Consideration should be given to inclusion of any *in vivo* evaluations as additions to general toxicity studies, to the extent feasible, in order to reduce animal use. In addition, primary pharmacodynamic studies (*in vivo* and/or *in vitro*) are intended to investigate the mode of action and/or effects of a substance in relation to its desired therapeutic target. Such studies are generally conducted during the discovery phase of pharmaceutical development and as such, are not generally conducted in accordance with Good Laboratory Practices (GLP). These studies can contribute to dose selection for both nonclinical and clinical studies.

Data from general pharmacology studies (USA, Japan, and EC) or safety pharmacology studies in the assessment of a marketing application have been accepted worldwide. The Japanese Ministry of Health and Welfare issued the Guideline for General Pharmacology in 1991. In this guideline, general pharmacology studies include those designed to identify unexpected effects on organ system function and to broaden pharmacological characterization (pharmacological profiling). However, there has been no internationally accepted definition of the terms *primary pharmacodynamic*, *secondary pharmacodynamics*, and *safety pharmacology*. The need for international harmonization of the nomenclature and the development of an international guidance for “safety pharmacology” has been recognized.

Safety pharmacology uses the basic principles of pharmacology in a regulatory-driven process to generate data to inform risk/benefit assessment. The key issues are detection of an adverse effect liability, projection of the data into safety margin calculation and finally clinical safety monitoring. The creation of safety pharmacology has not resolved all challenges, especially with respect to detection of rare and lethal adverse effect liability [5].

One of the key roles of safety pharmacology is to help make the decision to begin testing in humans. Pharmacology alone does not define the fate of a new drug. The issues determining the point at which it is ethical to proceed with clinical trials inform a risk/benefit assessment that is weighed against clinical development costs and the potential market. Determining the risk/benefit ratio is especially difficult when rare, but potentially lethal events are a concern for a drug, which is intended for use against a non-life-threatening condition. The key point to emphasize is that just as preclinical discovery, studies never prove that a drug will be effective in patients. Thus the point at which preclinical data is sufficient to inform a decision on whether or not to proceed with a drug into clinical investigation is subjective and a matter of judgment (both for the company and for the regulators who scrutinize the application). The sooner a decision is made to kill a drug the sooner the company can begin to strategize on development pathways, that is, either develop another drug backup using a similar chemical scaffold or consider a dissimilar drug class or program. Investigation studies, each of which addresses a related hypothesis, take place after drug approval and extensive human exposure.

Basic Considerations

Administration route and frequency – The intended human route should be employed if possible. Usually, a single route of administration is sufficient.

Dose levels – Generally, three doses are chosen. The low dose is equal to or slightly higher than the dose that produces the primary pharmacological actions. The intermediate and high doses are chosen in order to establish a possible dose-response relationship.

Species, sex – For each test an appropriate animal species is chosen according to any background data related to the pharmacological effects, to its (the species') acceptance as a predictor of pharmacological drug effects, and to the recognition by regulatory authorities that the species is acceptable for pharmacodynamic studies. Use of rats and dogs is recommended because these species are the most commonly used in toxicological evaluations, and pharmacokinetic and toxicokinetic data are often available in these species.

Experimental design – The number of animals per group is chosen on the basis of the variability of measured parameters in order to test the absence or presence of examined effects using appropriate statistical tests.

The FDA and other governmental regulatory agencies have drafted guidelines for safety pharmacology that have been issued in draft form [6]. It should be emphasized that there is no official viewpoint as to the necessity of studies on most organ systems or how they are to be done. Requirements are now basically limited to adverse effects on the cardiovascular system, specifically of potential effects on arrhythmias. There is not now any specific testing recommended for safety related to the gastrointestinal system. Alternative approaches may be used if such approaches satisfy the requirements of the applicable statutes and regulations.

REGULATORY GUIDANCE DOCUMENTS (CURRENT VERSIONS)

The primary reference documents for safety pharmacology are found below:

ICH S7A, which was followed by many key regulatory documents which either focus on or mention safety pharmacology:

ICH S6(R1): Preclinical safety evaluation of biotechnology-derived pharmaceuticals.

ICH S7A: Safety pharmacology studies for human pharmaceuticals.

ICH S7B: Nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals.

ICH S9: Nonclinical evaluation for anticancer pharmaceuticals.

ICH M3(R2): Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals.

ICH E14: Clinical evaluation of QT/QTc interval and proarrhythmic potential for non-antiarrhythmic drugs.

EMA: EMEA/CHMP/SWP/94227/2004. Adopted by CHMP. Guideline on the Non-Clinical Investigation of the Dependence Potential of Medicinal Products.

FDA U.S. Department of Health and Human Services Food and Drug Administration
—Center for Drug Evaluation and Research (CDER). Guidance for Industry.
Assessment of abuse potential of drugs. Final Guidance.

FDA U.S. Department of Health and Human Services Food and Drug Administration
—Center for Drug Evaluation and Research (CDER). Guidance for Industry.
Exploratory IND studies.

The specific studies that should be conducted and their design will vary based on the individual properties and intended uses of the pharmaceuticals. General principles for safety using scientifically valid methods should be used, and when there are internationally recognized methods that are applicable to pharmaceuticals, these methods are preferable.

The main object of safety pharmacology studies is to identify undesirable pharmacological and pharmacodynamic properties of a substance that may have relevance to its human safety. To evaluate adverse pharmacodynamic and/or pathophysiological effects of a substance observed in toxicology and/or clinical studies, and investigate the mechanism of the adverse effects observed regardless of the route of administration, exposure to the parent substance and its major metabolites should be similar to, or greater than, that achieved in humans when such information is available. Assessment of effects by more than one route may be appropriate if the test substance is intended for clinical use by more than one route of administration (e.g., oral and parenteral), or where there are observed or anticipated significant qualitative and quantitative differences in systemic or local exposure.

Generally, any parent compound and its major metabolites that achieve, or are expected to achieve, systemic exposure in humans should be evaluated in safety pharmacology studies. Evaluation of major metabolites is often accomplished through studies of the parent compound in animals. If the major human metabolites are found to be absent or present only at relatively low concentrations in animals, assessment of the effects of such metabolites on safety pharmacology endpoints should be considered. If metabolites from humans are known to substantially contribute to the pharmacological actions of the therapeutic agent, it could be important to test such active metabolites. When the *in vivo* studies on the parent compound have not adequately assessed metabolites, the tests of metabolites can be used in *in vitro* systems based on practical considerations. *In vitro* or *in vivo* testing of the individual isomers should also be considered when the product contains an isomeric mixture.

The following are some situations in which safety pharmacology may not be required:

1. Locally applied agents (e.g., dermal or ocular) where the pharmacology of the test substance is well characterized, and where systemic exposure or distribution to other organs or tissues is demonstrated to be low.
2. Safety pharmacology studies prior to the first administration in humans may not be needed for cytotoxic agents for treatment of end-stage cancer patients. However, for cytotoxic agents with novel mechanisms of action, there may be value in conducting safety pharmacology studies.

3. Biotechnology-derived products that achieve highly specific receptor targeting are often sufficient to evaluate safety pharmacology endpoints as a part of toxicology and/or pharmacodynamic study; therefore, safety pharmacology studies can be reduced or eliminated for these products.
4. Biotechnology-derived products that represent a novel therapeutic class and/or those products that do not achieve highly specific receptor targeting; a more extensive evaluation by safety pharmacology studies should be considered.
5. Additional exceptions where safety pharmacology testing is not needed; for example, in the case of a new salt having similar pharmacokinetics and pharmacodynamics.

Gastrointestinal Safety Studies

Effects on the gastrointestinal tract are not considered to be of primary importance based on a hierarchy of organ systems. Vital organs or systems, the functions of which are acutely critical for life (e.g., the cardiovascular, respiratory, and central nervous systems), are considered to be the most important ones to assess in safety pharmacology studies. Other organ systems (e.g., the renal or gastrointestinal system), the functions of which can be transiently disrupted by adverse pharmacodynamic effects without causing irreversible harm, are of less immediate investigative concern.

The gastrointestinal tract extracts nutrients, electrolytes, minerals, and water, and is prone to injury as a result of oral drug administration. Clinical assessment of the GI tract is often limited to measurements of transit time and observations of vomiting or diarrhea, despite the existence of methods and techniques capable of assessing specific changes in GI function at the membrane, cell, and whole animal levels. In some specific cases, membrane studies, measurement of the uptake of solutes, and electrolyte transport, assessing the effects of compounds on trans-epithelial GI transport and flux may be required, but is not considered essential prior to the initial human trials. Such methods lend themselves to permeability, immunohistochemistry, morphology, and molecular biology techniques. In anesthetized animals, ligated segments of the intestine can be infused with test compounds, providing information about absorptive and secretory processes important for the treatment of diarrhea. Finally, advances in the field of imaging, combined with endoscopy, have resulted in a wireless capsule, allowing the inspection of the GI tract anatomy and pathology without surgical intervention.

The guidelines suggest evaluation on gastric secretion, gastrointestinal mucosal injury potential, bile secretion, and intestinal transit time. The investigator has discretion on which testing shall be used depending on the class of pharmacological agent being developed. The tests recommended, but not required, to provide important information on safety and potential side effects, including gastric ulcerogenicity (rat), gastric acid secretion, (rat), gastric emptying (rat), fecal pellet output (mice or rats), emesis (ferret), intestinal transit (mice or rats), and biliary secretion (rat), pancreatic secretion (rat). This chapter will not consider *in vitro* organ bath systems since they do not appear to offer useful additional information in most cases.

General recommendations for GI safety study design

Acceptance by local animal use committee—All methods used should be approved by the appropriate animal care committee.

Protocol—A detailed protocol should be written and used for all safety pharmacology studies. All parameters should be spelled out.

Dose range testing—A dose range starting at the “therapeutic” dose and increasing at multiples of 2–5 for at least 3 doses.

Choice of test animal—If possible, an animal similar (species, sex, and source) to that in which efficacy has been shown should be used.

Test material—The test material should be similar to that shown to be active (the same batch, if possible). Care should be taken to use the same vehicle, salt, and method of preparation.

Time course—The duration of the study should be based on information derived from efficacy studies.

Good Laboratory Practice—If required, the necessary SOPs and protocols should conform to GLP and have all necessary approvals and audits.

ANIMAL MODELS FOR EVALUATION OF SAFETY PHARMACOLOGY

The following will describe animal models available and accepted for evaluation of safety pharmacology on the gastrointestinal tract. Most of these methods are straightforward and do not require extensive equipment or surgical skills. They have been chosen on the basis of reported usage in the literature and personal experience [7].

Mouth

Salivary Secretion

Although there are no suggestions that dry mouth (xerostomia) is an important adverse effect it is an important cause for non-compliance with many chronically administered drugs. Xerostomia also known as salivary gland hypofunction, is an uncomfortable oral symptom that results from decreased secretion of saliva.

Classes of Compound with Potential Liability and Possible Mechanisms

Drugs that have the potential to cause xerostomia include antiarrhythmics, anticholinergics, antihistamines, antihyperlipidemics, anti-inflammatory agents, antiulcer agents, coronary vasodilators, drugs for parkinsonism, and psychotropic drugs. Xerostomia can have a major effect on a patient's nutritional status since lack of saliva can make it difficult for the patient to speak, taste, chew, and swallow food.

Drug-induced xerostomia may or may not involve changes in neural control of salivation. For example, diuretic-induced dehydration can induce xerostomia.

Drug-induced xerostomia is potentially reversible, as the drugs usually do not cause permanent damage to the salivary glands.

Animal Models Available

Measurement of Salivary Secretion

Mice: Mice are anaesthetized with pentobarbital intraperitoneally [8]. Any saliva remaining in the oral cavity is removed with a cotton ball before measuring the total saliva collected in the cavity for a 10-min period. The saliva is absorbed onto three to five cotton balls for 10 minutes and the balls weighed on an electric balance immediately after the collection period to prevent moisture loss. To examine the effects of oral administration of a test agent on pilocarpine-induced salivary secretion, pilocarpine is administered intravenously 0.5, 2, 6, 12, and 24 hours after oral administration of the drugs, and saliva collected for 10 minutes.

Rats: Rats are dosed with vehicle or test drug and are anesthetized with IP urethane [9]. Saliva in the animal's oral cavity was removed using an aspirator immediately before drug challenge. Saliva is successively aspirated at 1-minute intervals for 5 minutes after IV administration of secretagogues. The amount of saliva collected during each 1 min interval was quantitated by weight.

ESOPHAGUS

Erosion of the mucosa of the esophagus has not been a common problem with orally administered drugs, because the time the drug is in contact with the esophageal mucosa is usually limited. With liquid preparations, unless the compound has corrosive activity, clearance of the formulation is usually rapid. However, there have been some circumstances in which transport through the esophagus is delayed due to pathophysiology or disintegration of the formulation in the esophagus in the lumen, releasing a potentially damaging substance. Problems can also occur if the patient is supine and transport along the esophagus is delayed.

Classes of Compounds and Mechanisms

Drug-induced esophageal injury can occur with the current use of some formulations of oral drugs. Non-chewable tablets or capsules usually pass quickly through the esophagus and release their contents in the stomach or lower in the intestine. Occasionally, these tablets and capsules can get lodged within the esophagus, dissolve, and release their concentrated contents, causing direct mucosal damage. An important warning sign is a dull, aching pain in the chest or shoulder after taking the drug.

The severity of drug-induced esophageal damage ranges from mild, asymptomatic inflammatory changes to severe ulceration and stricture formation. The acute forms are the most common, are self-limited, and do not lead to serious medical problems.

Many factors influence the severity of drug-induced injury to the esophagus. Chemical and physical properties of the drug, such as its pH, concentration of medication, drug formulation, and size and shape of the tablet or capsule. Other factors include delay in transit time of the drug and duration of contact with esophageal mucosa. When gelatin capsules are administered with inadequate liquid, they may become sticky as they dissolve and delay transit time of the drug.

Almost 100 different drugs are known to cause esophageal damage [10]. Aspirin, tetracycline, quinidine, potassium chloride, vitamin C, and iron can all cause esophageal ulcers. Alendronate as well as other bisphosphonates can cause esophagitis, including severe ulcerations. Patients should take alendronate and other drugs in its class with at least 180 mL of water and remain in an upright position for at least 30 minutes before eating to prevent esophageal ulceration. Bisphosphonates are a local irritant to the gastrointestinal tract. The comparable molecular structure of bisphosphonates and phosphatidylcholine create competitive binding on the gastrointestinal lining. When bisphosphonates bind, the hydrophobic barrier is destroyed, and gastric acid is able to reach the epithelium resulting in irritation.

Animal Models Available

Acute dosing in rats with a solution or suspension of test agent is not a suitable model. Esophageal ulcerogenicity is rarely evaluated in animal studies, since test compounds are usually delivered directly into the stomach via gavage. A test using the anesthetized dog has been described [11]. An endotracheal tube is passed into the esophagus, with the cuff inflated directly caudal to the larynx. A rubber catheter is then passed through the tube, extending caudally just past the end of the tube. An infusion pump delivers the test liquid through the catheter over a 30-minutes period. The cranial portion of the dog is elevated, so that the liquid would flow aborally. The infusions are generally administered for 5 consecutive days, and the dogs euthanized on the fifth day for gross and histopathologic examination of the esophagus. For delivery of compounds in tablet form, a clear tube can be passed into the cranial third of the esophagus. The tablet, tied to a premeasured length of silk suture, is then introduced into the esophagus through the tube by using endoscopic retrieval forceps. The tablets are left in place for 1 hour, and the dogs are then euthanized and examined. These techniques allowed for accurate and reliable testing in dogs of the esophageal irritancy of compounds in either the liquid or tablet form.

Endoscopy may also be useful for evaluation of acute dosing with a capsule or tablet formulation administered to a dog followed by esophageal and gastric endoscopy. A dog model to examine the possible mechanism for the esophageal adverse events has been published and showed that under low pH conditions, alendronate sodium can cause esophageal irritation [12].

Gastroesophageal Reflux (GER)

GER occurs as a result of excessive exposure of the esophageal mucosa to the acidic gastric contents. The signs and symptom range in severity from heartburn

to severe erosive esophagitis. The etiology of GER is multifactorial. Contributing factors include an atonic lower esophageal sphincter (LES), hiatal hernia, impaired motility of the esophagus, lessened resistance of the esophageal epithelium to injury, increased gastric secretion, and delayed gastric emptying.

Normally, the LES, with a zone of pressure of 15–35 mm Hg, prevents the gastric contents from entering the esophagus. Some drugs (e.g., anticholinergics, calcium channel blockers, ethanol, and nitrates) cause gastroesophageal reflux by inappropriately relaxing the LES. Progesterone, theophylline, and tricyclic antidepressants also reduce LES pressure.

Animal Models Available

Lower esophageal sphincter pressure may be measured in the anesthetized ferret using a perfused esophageal tube [13]. Ferrets are anaesthetized with isoflurane (4% induction with 2% maintenance) using a facial mask. The jugular vein and carotid artery are cannulated for intravenous drug and fluid administration and for monitoring of arterial blood pressure, respectively. Anesthesia is administered during surgery through a tracheal cannula which was also used to monitor respiration rate via a thermoprobe, which measured changes in temperature caused by inhalation and exhalation during the respiratory cycle. A miniaturized manometric assembly (Dent sleeve) was inserted perorally and positioned so that the sleeve lay across the LES. Esophageal, LES, and gastric pressures are recorded.

STOMACH

One of the primary sites for the occurrence of adverse effects of drug is on gastric mucosa and that drugs could produce gastric upset, and in some cases, lesions and ulcerations leading to bleeding.

Classes of Compound with Potential Liability and Possible Mechanisms

Any class of compound may produce gastric damage. The propensity to injure the gastric mucosa, which under normal conditions is resistant to acid conditions and the presence of proteolytic enzymes, is usually dependent on their ability to modify the protective barrier. This allows the acid and enzymes to contact mucosal cells lacking the protective coating of mucous. Drugs that are organic acids have detergent action or effect the levels of protective prostaglandins are all suspect. Borsch and Schmidt [14] published a survey of the damaging effect of acetylsalicylic acid, non-steroidal anti-inflammatory drugs and corticosteroids on the gastroduodenal mucosa. They showed that these effects could be quantified in humans by blood loss studies, histology, and endoscopy. They point out the pathophysiology of these lesions and stress the cytoprotective role of endogenous prostaglandins. This and other epidemiologic data strongly support an association between frequent and

heavy intake of acetylsalicylic acid and other related compounds to gastrointestinal bleeding and gastric ulcer, whereas the association with duodenal ulcers is far less clearly established.

Aspirin and the then new class of non-steroidal anti-inflammatory agents were found to produce significant and predictable lesions after a single dose in the fasted rat only [15]. Other agents such as alcohol and steroids were also shown to have similar effects. It was not until 1971 when Vane and his associates [16] showed that the mechanism of action of aspirin and NSAIDs was due to inhibition of prostaglandin formation and that these were essential to the integrity of the upper gastrointestinal mucosa [17]. Cyclooxygenase (COX) leads to the formation of prostaglandins (PGs) that cause inflammation, swelling, pain, and fever. By inhibiting this key enzyme in PG synthesis, aspirin-like drugs also prevented the production of physiologically important PGs, which protect the stomach mucosa from damage by hydrochloric acid, maintain kidney function, and aggregate platelets when required. This conclusion provided a unifying explanation for the therapeutic actions and shared side effects of the aspirin-like drugs. More recently with the discovery of a second COX gene, it became clear that there are two isoforms of the COX enzyme. The constitutive isoform, COX-1, supports the beneficial homeostatic functions, whereas the inducible isoform, COX-2, becomes up-regulated by inflammatory mediators, and its products cause many of the symptoms of inflammatory diseases such as rheumatoid and osteoarthritis. Compounds developed that had selective COX-2 inhibitory activity were found to have potent analgesic and anti-inflammatory action but with less propensity to produce gastrointestinal mucosal damage.

Oral administration of indomethacin and other NSAIDs produce hemorrhagic gastric lesions in both normal and arthritic rats, although the severity of lesions was significantly greater in the latter group. In contrast, neither rofecoxib nor celecoxib caused any gastric damage in normal rats, but both drugs provoked hemorrhagic gastric lesions in arthritic rats. The expression of COX-2 mRNA and immuno-positive cells was observed in the gastric mucosa of arthritic but not normal rats. The gastric mucosal prostaglandin (PGE₂) content was significantly elevated in arthritic rats in a rofecoxib-sensitive manner [18]. Since COX-2 inhibitors produce gastric lesions in arthritic rats, similar to the nonselective COX-inhibitors, it may be important to test potential anti-inflammatory agents in both normal and arthritic rats to evaluate the true gastric mucosal toxicity. COX-2 is up-regulated in the stomach of arthritic rats, and PGs produced by COX-2 play a role in maintaining the integrity of the gastric mucosa. Dose-dependent administration of celecoxib and rofecoxib as COX-2 inhibitors and non-COX-1 inhibitors, respectively, did not produce toxic injuries on healthy gastrointestinal mucosa, thus providing a broad therapeutic spectrum [19]. On the other hand, when administered in the presence of altered gastrointestinal mucosa, they worsened and complicated gastric ulcers, and also induced necrosis in the small intestine, thereby restricting their clinical use. At this time, the chronic use of NSAIDs, whether COX-1 or -2 inhibitors, are under attack due to an apparent increase in cardiac-related deaths in patients on high doses. It is not

clear at this time whether the use of COX-2 inhibitors in patients susceptible to gastrointestinal pathology is a risk worth taking.

Ethanol is a well-known gastric mucosal irritant. Ethanol (80% v/v, po.) given repeatedly to rats induces sub-chronic gastritis [20]. It can also produce gastric lesions after a single dose. Sub-chronic gastritis potentiated gastric ulcer formation, such as that produced by acetic acid. The induction of gastritis resulted in an activation of TNF alpha expression followed by apoptosis in the gastric mucosa. This could lead to an increase in the severity of ulcerative damage in the stomach. It may be useful to test therapeutic agents likely to be given to humans with gastritis in a mouse with sub-chronic gastritis.

Animal Models Available

Fasted Rat: Testing is usually simple and consists of oral dosing in 18- to 24-hour fasted rats and sacrificing after 3–5 hours. The stomachs are removed and either filled with saline or formalin. Then the mucosa is inspected, usually under low magnification, for the presence or absence of hemorrhagic spots, lesions, and ulceration. There have been numerous attempts to use scores and automated methods to provide quantitative data. However, in general, most investigators are interested in whether their compound produces a significant degree of mucosal damage and how this dose relates to the dose producing a therapeutic effect.

There are multiple factors influencing the rat model and its usefulness in predicting activity in humans [21]. The most important factor in using this model is to insure a fasting period with a sufficient duration so that the stomach is devoid of food or ingested feces. Presence of material in the gastric content will interfere with observation of gross lesions and hemorrhagic spots and may provide enough buffering to raise the pH of the stomach above four.

Testing should be done for all clinical candidates that are to be administered orally. Gastric irritation is such a common side effect that this information should be available prior to the first oral administration of an experimental drug to humans. Clinical manifestations of drug-induced stomach due to medication type, dose, and whether associated with stomach irritation.

Gastric Acid Secretion

Although guidelines suggest that the effect of experimental drugs should be evaluated for effects on gastric acid secretion, this test does not appear to be needed for most classes of compounds.

Classes of Compound with Potential Liability and Possible Mechanisms

Compounds that appear to be similar to known gastric antisecretory agents, muscarinic agonist or antagonists, and histamine agonists and antagonists should be tested to evaluate potential effects on acid secretion [22].

Animal Models Available

FDA guidelines suggest measurement of gastric acid secretion as one of the suggested baseline preclinical tests. Although it is not helpful in predicting potential side effects it could provide some mechanistic evidence of the mechanism of action of the test compound. There are several models available for evaluation in the intact un-anesthetized animal, which is the only appropriate approach.

Pylorus ligated (Shay) rats: Rats are fasted for 36 hours with access to water ad libitum before the pylorus was ligated under ether anesthesia, care being taken not to cause bleeding or to occlude blood vessel. Test drugs are usually administered orally but may be given by other appropriate routes. The animals are sacrificed at 6 hours after the pylorus ligation, the stomachs removed, and contents collected. The volume of collected gastric secretion is determined and analyzed for titratable acidity against 0.01 mol/L sodium hydroxide to pH 7. Total acid output is calculated then calculated by multiplying the concentration of acid by the volume [23].

Gastric Fistula Models – Komarov et al. [24] described a chronic gastric fistula rat preparation. An acute gastric fistula rat model was described by Jacoby et al. [25]. One acute gastric fistula preparation has been described in which a flanged plastic tube is inserted into the gastric lumen under anesthesia. The rat allowed to recover from surgery, and gastric juice collected for 1–3 hours. Gastric acid secretion is about 50% of maximum so that both inhibitory and stimulatory effects may be observed. If another species is required, the chronic gastric fistula dog may be used [26]. The dog does not secrete acid in the fasted state, so that it is the most sensitive for evaluation of compounds with potential acid secretory stimulatory activity. If antisecretory activity needs to be evaluated in the dog, the test compound must be tested against gastric acid stimulated by gastrin, histamine, or other acid secretory agent.

SMALL INTESTINE

The small intestine is also a common site for non-steroidal anti-inflammatory drug (NSAID) toxicity. Although NSAID strictures and perforation are relatively rare in humans, two-thirds of regular NSAID users may be prone to small bowel enteropathy. With the use of a capsule camera, it is now possible to visualize the whole small intestine in humans. This will allow clinical trials to include evaluation for the potential of small intestinal pathology. Previously, only limited visualization could be done using a flexible endoscope.

Classes of Compound with Potential Liability and Possible Mechanisms

NSAIDs and potassium supplements are the primary causes of drug-induced lesions. Enteric-coated potassium chloride was formulated to resist the acidic environment of the stomach. However, when the enteric-coated tablets dissolve in the small intestines, a high concentration of ionized potassium accumulates adjacent to

the dissolving tablet and causes direct epithelial damage and mucosal ischemia as a result of local vasoconstriction. These changes in the epithelium and mucosa lead to ulceration, bleeding, or perforation. In addition, a stricture may develop secondary to fibrosis associated with the healing process. To prevent this damage, the formulations of potassium chloride tablets were changed to a controlled-release wax matrix system and to microencapsulation to reduce the risk of injuring the gastrointestinal mucosa.

Cytotoxic Effects

Many chemotherapeutic drugs have cytotoxic effects on mucosal cells of the small intestine because these cells have a high turnover rate. Chemotherapeutic drugs such as actinomycin D, bleomycin, cytosine, arabinoside, doxorubicin, 5-fluorouracil, methotrexate, and vincristine can cause erosive enteritis. The clinical features include pain, bleeding, vomiting, ileus, and diarrhea.

DIARRHEA

Drugs induce diarrhea by disrupting the normal physiologic processes that regulate fluid absorption and secretion, by altering defense mechanisms, and by damaging the mucosa of the small and large intestine.

Specifically, drugs can cause diarrhea by interfering with normal physiologic processes that play a role in fluid and electrolyte balance within the GI tract. Drugs can interfere with the $\text{Na}^+\text{-K}^+$ pump that regulates the active transport of water and electrolytes across the cell membrane. A drug can inhibit the enzyme $\text{Na}^+\text{-K}^+\text{-ATPase}$ so that energy cannot be released from adenosine triphosphate (ATP). The inhibition of the ileal and colonic $\text{Na}^+\text{-K}^+$ pump causes decreased fluid absorption and diarrhea.

Animal Models Available

Fed Rat: The rat is the usual model for evaluation of small intestinal lesions, at least those due to NSAIDs. Drug is administered to a fed rat with *ad lib.* access to food and water and observed for 2–3 days. Indomethacin and other NSAIDs will produce significant lesions and even perforations in the small intestine [27]. The fed rat was 8 times more susceptible to intestinal damage than the fasted rat. The 24-hour fasted rat was 13 times more susceptible to gastric damage than rats allowed access to food.

The only model useful for specific evaluation of potential damage to small intestinal and colonic mucosa was published by Fara [28]. He used an *in situ* rabbit colon or intestine model as a sensitive and reproducible test to evaluate the topical effect of up to three substances applied to the colonic mucosa, and found that doxycycline hyclate tablets and propranolol hydrochloride tablets produced macroscopic and histologic damage. Potassium released from Slow-K and Micro-K Extencaps

caused more irritation than from controlled-release KCl. This model may be useful in evaluating sustained or enteric coated formulation for potential lesion-producing activity and may be the only suitable way of evaluating potential problems with formulated products, which are too large to be dosed to most laboratory animals. The fed rat can also be used to evaluate the diarrheogenic potential by observing fecal pellet.

All compounds believed to have anti-inflammatory activity or have effects on cyclooxygenase should be tested [29]. Because of problems in humans (ischemic colitis) with at least one serotonin₃ antagonist, alosetron, this type of compound should also be evaluated [30,31]. Alosetron is a potent and selective serotonin antagonist that became the first FDA-approved agent for diarrhea-predominant irritable bowel syndrome. Since approval, significant side effects have been noted with its use, including severe constipation, fecal impaction, and ischemic colitis. Clinical, endoscopic, and pathologic features of the focal colitis strongly suggested ischemia and were observed in patients who should not have received the compound. Symptoms correlated temporally with alosetron use, and symptoms abated with discontinuation of the drug [30–32].

Gastrointestinal Propulsive Motility

Gastric Emptying

Although gastric emptying specifically is not required according to guidelines, the effect of experimental compounds on gastric emptying is important.

Classes of Compound with Potential Liability and Possible Mechanisms

Drugs affecting gastric emptying may cause a variety of objectionable side effects that can be evaluated early in the discovery process. Compounds affecting the autonomic nervous system (agonists or antagonists), drugs having serotonergic (agonist or antagonist activity), and a wide variety of compounds may have profound effects on gastric emptying. Drugs influencing emptying can also result in significant effects on pharmacokinetics, either increasing or decreasing blood levels achieved after oral administration.

Animal Models Available

The charcoal meal test is usually specified for evaluation of gastrointestinal propulsive motility. Unfortunately, it is not a good model for evaluation of gastric emptying, and can only provide an indirect indication of effects on gastric emptying. There are many specific test models to evaluate effects on solid or liquid emptying. When deciding what test to use it is important to remember that drug activity on solid and liquid meals may differ.

Liquid Meals

Emptying of a non-nutrient liquid solution of a non-absorbable dye in conscious rats (phenol red, carmine red) is a common method. Gastric emptying is measured at different time points after administration of a semi-viscous liquid containing a non-absorbable dye (phenol red or carmine red) as the marker. The volume of gastric emptying is calculated from the amount of dye remaining in the stomach, taking into consideration the volume of gastric secretions. Depending on the duration of the test, this method may be optimized for evaluation of agents augmenting (10–20 minutes) or inhibiting gastric emptying (30–90 minutes). This may also be done in chronically cannulated rats fitted with stainless steel gastric cannulas. A method using these has been described that can be utilized to evaluate gastric emptying using a non-absorbable dye [33].

An example of a typical study may be found in a study by de Rosalmeida et al. [34]. Phenol red dissolved in water is administered in a small volume to fasted rats. After test agent administration, they were gavaged (1.5 mL) with a test meal (phenol red in 5% glucose solution, 0.5 mg/mL) and sacrificed 10, 20, or 30 minutes later, and the volume of gastric contents determined by analysis of the dye remaining in the stomach. This was compared to vehicle-treated animals. In this study sildenafil delayed gastric emptying of a liquid meal. Phenol red has also been used in mice (0.15 mL/mouse) [35].

Feldman and Putcha [36] studied the activity of atropine sulfate, trihexyphenidyl HCl, benztropine mesylate, diphenhydramine HCl, and ethopropazine HCl on gastric emptying and intestinal transit of a phenol red solution in the rat. Inhibition of gastric emptying and intestinal transit were only observed after single and multiple oral doses of diphenhydramine and ethopropazine.

Charcoal Meal

Lotti et al. [37] described a simple test in the mouse used to evaluate CCK antagonists based upon visual determination of the gastric emptying of a charcoal meal. CCK-8, but not various other peptide and nonpeptide agents, effectively inhibited gastric emptying in this test system.

Drug Absorption

The use of absorption of a compound known not to be absorbed from the stomach, but having consistent duodenal absorption characteristics, has been used to quantify gastric emptying of a liquid meal [38]. The usual compound used is acetaminophen, since it is not absorbed through the gastric mucosa and blood levels are only observed after the compound reaches the duodenum. An oral dose of acetaminophen (100 mg/kg) is administered and blood samples collected before and up to 12 hours after administration. Plasma assays are performed using a high-performance liquid chromatography method with UV detection. The calculated

population pharmacokinetic parameters were K_a , K_{el} (first-order absorption and elimination constants), and V_d/F (apparent volume of distribution) provided information on the effects of drugs on transport of acetaminophen into the duodenum compared to vehicle controls.

Radioisotopes

Emptying of liquid nutrient meals tagged with a radioisotope has been used as a model in animals and is a common method in humans. This is a useful method for defining regulation of gastric emptying of nutrient liquid meals in mice. In larger animals a gamma camera may be used to quantify radioactive material remaining in the stomach. Solutions of non-absorbable radioisotopes such as radiolabeled chromium have been used for estimating gastric emptying. In one study, results were compared to emptying of a charcoal meal. Gastrointestinal propulsive motility was assessed in rats 15 minutes after intragastric instillation of a test meal containing charcoal (10%) and $\text{Na}^{251}\text{CrO}_4$ (0.5 microCi/mL) [39]. Gastric emptying was determined by measuring the amount of radiolabeled chromium contained in the small intestine as a percentage of the initial amount received. Then, gastrointestinal transit was evaluated by calculating the geometric center of distribution of the radiolabeled marker.

BEADS OR PELLETS

The use of beads or non-dissolving tablets to evaluate the effect of drugs on gastric emptying has been established as a method for discovery of agents with gastroprokinetic activity. Jacoby and Brodie [40] published a study using 1 mm amberlite pellets in rats and small enteric coated barium tablets, tracked by fluoroscopy in rhesus monkeys to evaluate gastric emptying produced by metoclopramide. Other variations of the use of small pellets (polystyrene, plastic, glass) have been used to evaluate drug effects on gastric emptying [41,42]. Twenty-four-hour fasted rats are dosed with the test compound at the appropriate route and dose. A set number of pellets are administered by an appropriate size gavage tube and after 10–60 minutes the rat is euthanized, and the stomach carefully removed. The number of beads remaining in the stomach is counted and compared to a group of rats receiving the vehicle control. The experiment can be designed to detect agents increasing gastric emptying (shorter duration) or those inhibiting gastric emptying (longer duration). Some information as to intestinal propulsion can be obtained by counting pellets in segments of the small intestine. The study may also be done in rats with delayed gastric emptying due to streptozotocin-induced diabetes.

Asai et al. [43] studied the effect of clonidine on gastric motility by examining the effect on gastric emptying of indigestible solids. Clonidine or saline was injected intraperitoneally, and ten steel balls (1.0 mm in diameter) were inserted into the stomach. Gastric emptying was examined at 3 hours. Clonidine delayed gastric

emptying of the balls. Yohimbine, but not naloxone, significantly antagonized the inhibitory effect of clonidine.

Chow Meal

The use of a meal of ground rat or mouse chow may be useful in the estimation of gastric emptying of a semi-liquid meal. Differences in gastric weight between treated and control animals provide a rough index of emptying. Animals are deprived of food for 18–24 hours and allowed free access to preweighed solid chow for a set period of time (1–3 hours). The food is then removed, and gastric emptying can be determined over several hours by measuring the wet weight of the stomach. For a more accurate estimate, the ground meal can be labeled with a radioisotope that binds to the meal or using ground-up liver from an animal fed a radioisotope such as technetium.

GLUCOSE SOLUTION OR OTHER LIQUID MEAL

The emptying of a glucose meal or other meal containing a thickening agent or plain water may be quantitated by comparison of gastric weight after a set period of time. Although this may be affected by secretion into the gastric lumen, it provides a rough estimate of emptying of a liquid load [44].

Slowing gastric emptying can affect absorption of the agent and may be responsible for drug interactions by interference with the absorption of concomitantly taken medication. Inhibition of gastric emptying in the rat may also predict other adverse effects such as gastroparesis. Augmentation of gastric emptying may also affect the pharmacokinetics of co-administered agents and may also indicate a useful pharmacological action.

SMALL INTESTINAL PROPULSIVE MOTILITY

Classes of Compound with Potential Liability and Possible Mechanisms

Opioids and a number of other classes of agents have significant effects on small bowel propulsive motility. The charcoal meal test will usually pick up both stimulants and inhibitors of propulsion and is one of the tests recommended in the guidelines.

The motor activity of the small intestine is less likely to be affected by drugs than in the colon. However, drugs such as phenothiazines, antiparkinsonian agents, tricyclic antidepressants, anticholinergics, opiates, loperamide, and calcium channel blockers can also inhibit the motility of the small intestine. The reduction of small bowel motility may be severe enough that it can cause paralytic ileus or pseudo-obstruction.

Animal Models Available

Charcoal Meal

The charcoal meal test is the most commonly used test for effects on propulsive motility. It can be performed in the fasted rat or mouse. The charcoal meal has long been used as a measure of small intestinal propulsive motility since published by Macht and Barba-Gose in 1931 [45]. It is one of the recommended tests in most guidelines. The test agent is given by the appropriate route, and a small volume (depending on the size of the test animal) of non-activated charcoal suspended in 1%–2% carboxy or hydroxypropyl cellulose is administered orally. The test animal is euthanized at an appropriate time (1–3 hours) and the stomach and small intestine removed. The distance of the furthest sign of charcoal in the intestine is noted and compared to the total length of the small intestine (pylorus to ileocecal valve). The inhibitory effect of repetitiously administered loperamide, a peripheral mu-opioid receptor agonist and well-recognized antidiarrheal agent, on mouse gastrointestinal transit was compared with that of morphine in order to examine the development of tolerance to mu-opioid receptor agonist-induced constipation (antitransit effect) [46]. When administered subcutaneously 15 minutes before the oral injection of charcoal meal, loperamide (0.1–30 mg/kg) and morphine (1–8 mg/kg) dose-dependently and significantly inhibited gastrointestinal transit of charcoal with the ID₅₀ values of 1.6 and 3.6 mg/kg, respectively.

Other tests using radionuclides have also been used but require special laboratories for handling radioactive materials and do not provide significantly more information.

Drugs affecting small intestinal propulsive motility may possess either inhibitory or stimulatory activity on small intestinal propulsive motility. These tests are good predictors for evaluating the possibility of the test compound to have constipating or laxative activity.

COLONIC PROPULSIVE MOTILITY

The purpose of testing the effect of compounds on colonic motility is primarily to predict the potential for producing constipation or diarrhea. There are several simple tests available that have good correlation to clinical effects. They may also provide information as to mechanism of action and duration of action.

Classes of Compound with Potential Liability and Possible Mechanisms

All compounds in development should be tested for effects on intestinal propulsive motility. These tests provide a simple method for determining potential constipation or diarrhea as an adverse effect. This activity is a potential for many classes of compounds, and this test may provide information indicating a better or worse profile

in a series of compounds or show that it is better than reference compounds. There are many mechanisms that may manifest themselves as effects on intestinal propulsion.

Animal Models Available

Diarrhea and constipation are two of the most commonly observed side effects of therapeutic agents. The tests available are excellent at predicting the possibility in clinical studies and may be useful in early developmental studies in choosing a lead compound. All compounds should be tested in one or more of these tests prior to the first clinical trial.

Fecal Pellet Output

Monitoring the output of fecal pellets in mice or rats is one of the simplest tests available for evaluation of potential activity on intestinal propulsive motility. It can be useful for predicting constipation or diarrhea in mice or rats, although other species may be used. Test agent is administered by the appropriate route to rats or mice that are provided with access to food and water. Results may be monitored by counting fecal pellets and noting composition (solid, semisolid, not formed) or by weighing fecal output (ether wet or dry weight). Care must be taken to prevent loss of fecal pellets through the cage floor.

Drugs increasing colonic propulsion and excretion, either to activity on motility or increase fluid content (inhibition of absorption/ increase secretion), can be easily detected [47]. Serotonin and a serotonin₃ receptor agonist, 2-methyl-5-HT, dose-dependently increased fecal pellet output in conscious rats. The selective 5-HT₃ receptor antagonists GK-128, granisetron, ramosetron, azasetron and ondansetron depressed the increase in fecal pellet output caused by 2-methyl-5-HT. Granisetron and ramosetron dose-dependently reduced the spontaneous excretion of fecal pellets. Croci and Bianchetti [48] showed the effects of several alpha-adrenoceptor antagonists on fecal output and water content in rats. The rat colon appears to be under tonic inhibitory control of prejunctional alpha₂-adrenergic receptors, whose blockade by specific antagonists induces fecal excretion. The alpha_{2A}-receptor subtype appears to be the most likely candidate for controlling fecal excretion through inhibition of acetylcholine release.

Fecal pellet output has also been used for evaluation of potential antidiarrheal activity. Oral doses of castor oil increase both the fecal output (dry or wet weight) and the frequency of diarrhea in mice. Bismuth subsalicylate significantly prevented the enhancement of charcoal-meal transport induced by castor oil in both mice and rats [49]. Increased fecal output (dry or wet weight) and increased frequency of diarrhea in mice were significantly reduced by bismuth subsalicylate in a dose-related fashion. Blockade of castor oil diarrhea was also used to discover the first synthetic antidiarrheal agents by Awouters et al. [50]. The time-course of castor oil-induced diarrhea in fasted rats was quantified by weighing stools every 15 minutes for 8 hours after the challenge and then after 24 hours. Diarrhea began within 1 hour as a series of rapidly occurring evacuations over 20 to 40 minutes. Pretreatment with small doses

of loperamide caused a significant reduction. Riviere et al. [51] used Prostaglandin E_2 to produce diarrhea in mice. When given by intraperitoneal administration, PGE_2 induced a dose- and time-dependent diarrhea. This provides a convenient method for investigation of mechanism of action of antidiarrheal agents.

Glass Bead Expulsion

The expulsion of a glass bead inserted into the distal colon of rats or mice has been utilized as assay to investigate potential effects on colonic propulsive motility. This test is relatively specific for effects on propulsion and can detect most agents producing constipation in humans. It has been used to screen for antidiarrheal agents. In the rat, it may also be useful for evaluation of agents increasing or decreasing colonic propulsive motility. However, in the mouse, it is only suitable for agents inhibiting propulsion.

The test is simple to do and consists of inserting a glass bead (~2–4 mm) through the anus into the distal colon [52]. This test can detect compounds that have significant constipating activity or may be used as a primary screen for irritable bowel syndrome. A single 3-mm glass bead is inserted 2 cm into the distal colon of each mouse using a glass rod, one end of which was fire-polished so as to be rendered atraumatic. After bead insertion, mice are placed in individual plastic cages lined with white paper to aid visualization of bead expulsion. The time required for expulsion of the glass bead is determined to the nearest 0.1 minute for each mouse. Mice that did not expel the bead within 1 hour are necropsied to confirm the presence of the bead in the lumen of the large intestine. Mice for which bead localization could not be confirmed were not included in the results. Drugs interfering with expulsion may work by local effects in the distal colon blocking afferents, inhibiting smooth muscle propulsive motility directly or indirectly, through receptor-mediated effects on the nervous network in the colon, or through CNS-mediated effects. The rat provides a better model for detection of agents that increase propulsive motility since expulsion is much slower than the mouse. The test may be useful to evaluate laxatives having direct effects on propulsive motility such as $5HT_4$ agonists.

Studies using the glass bead have been used to evaluate mechanism of action. Since drugs may be administered ICV in mice, it has also been used to investigate the site of action when compared to peripherally administered compounds [53,54]. It has also been used to study the effect of peptides and opioids [55–57] radiowaves [58], and nociceptin [59].

Yamada and Onoda [60] have studied the colonic prokinetic activity of oral T-1815 and compared it with that of yohimbine and naloxone in mice by designing an experiment in which colonic propulsive motility is inhibited by clonidine (3–30 mg/kg s.c.) or loperamide (0.3–3.0 mg/kg s.c.). Yohimbine (0.3–10 mg/kg) and T-1815 (0.1–10 mg/kg) showed a dose-dependent reduction of the delay in evacuation induced by clonidine, but naloxone had no effect. The loperamide-induced retardation of colonic propulsion was reduced by naloxone (0.3–10 mg/kg) and T-1815 (0.1–10 mg/kg) in a dose-dependent manner, but yohimbine had no effect.

In normal animals, yohimbine and naloxone had no significant effect on evacuation, while a slight acceleration was observed with T-1815 at 10 mg/kg.

Drug-Induced Emesis

Nausea and vomiting are one of the most frequent adverse effects observed in clinical trials. Although emetic activity may be classified as a CNS-related side effect, emesis produces significant disturbance of gastrointestinal functions and may produce problems in short- and long-term safety evaluation in dogs or monkeys.

Classes of Compound with Potential Liability and Possible Mechanisms

All compounds related to known emetogenic agents should be tested. A wide variety of clinically useful compounds are associated with nausea and vomiting. Testing may provide information as to improvement of side effect profile compared to other similarly effective candidates.

Nausea and vomiting are common side effects of drugs, usually occurring early in the course of pharmacologic therapy. Among classes of compounds with known emetogenic activity are cytotoxic agents, dopamine agonists, and antidepressants.

Both the vomiting center (VC) and the chemoreceptor trigger zone (CTZ) play an important role in inducing vomiting. The vomiting center receives neural impulses from different sites in the body such as the CTZ and GI tract. Chemotherapy administration appears to induce vomiting by directly damaging cells in the GI tract. This is followed by the release of significant amounts of serotonin from enterochromaffin cells in the GI tract. When serotonin binds to serotonin (5-HT₃) receptors in the wall of the GI tract, neural impulses are sent to the VC.

Animal Models Available

Although not required in guidelines for inclusion of safety pharmacology, this test should be done on all clinical candidates prior to Phase I studies. The test is relatively inexpensive and does not require a great deal of test compound. Rats, mice, and guinea pigs are not capable of vomiting so initial studies in models do not provide information concerning emetogenic activity. The dog has been the standard model for testing; however, this may be a problem in early development due to expense and supply of material. Yamada and Onoda [60] has reviewed animal models including nonhuman primate, dog, cat, and ferret. The categories of pharmacologic compounds include both those compounds that act on identified membrane receptors (e.g., cholinergic agonists, catecholamines, and neuroactive peptides) and those that act on unidentified receptors (e.g., cardiac glycosides and veratrum alkaloids). Emphasis is placed on emetic dose-response relations and threshold ED₅₀ and ED₁₀₀ values calculated from these relations, as indices of species sensitivity to emetic stimuli.

Several alternatives to the use of dogs have been suggested. One, the house musk shrew, has been used; however, it was not found to be sensitive to many well-known

emetic agents, such as morphine. In fact, opioids had anti-emetic activity [61]. There are few references to its use and its validity, as a test, has not been proven.

Ferret: The ferret has been shown to be an excellent model for screening anti-emetics and is being used extensively in the development of anti-emetics useful against chemotherapy and radiation-induced emesis. The use of ferrets to evaluate either emetogenic or anti-emetic activity has been documented [62]. There appears to be a good correlation between emetogenic activity in the ferret and activity in humans. Animal models of chemotherapy/radiotherapy-induced emesis successfully predicted the clinical efficacy of the 5-HT₃ receptor antagonists for the control of acute emesis [63]. Further studies in animals have provided valuable information relating to the pathophysiology of emesis and the mechanism of action of 5-HT₃ receptor antagonists. These agents inhibit emesis by blocking the action of 5-HT at 5-HT₃ receptors on the vagus nerve in the gastrointestinal tract and in the hindbrain vomiting system. Serotonin is believed to be released from enterochromaffin cells following cytotoxic therapy or radiation. The mechanism by which 5-HT is released from enterochromaffin cells is unknown and, although various mechanisms have been proposed, none of these have provided convincing supportive evidence.

Emetics may produce acute or delayed vomiting. This is specifically true for cytotoxic agents similar to cisplatin. Ferrets, when given a dose of cisplatin and observed for 3 days, show a pattern of emesis similar to that seen in humans with two distinct phases of emesis, an acute phase and a delayed phase. 5HT₃ antagonists are effective in reducing the emetic response over days 1–3. When a higher dose of cisplatin is used, emesis usually occurs in less than 2 hours. This can also be blocked by serotonin antagonists.

The typical emesis study is done using male descended and castrated ferrets. The ferrets are individually housed in stainless steel cages and provided ferret diet and water *ad lib*. Dosing may be done in either overnight fasted or fed ferrets. After oral dosing, ferrets are then observed for signs of lip licking, malaise, retching, vomiting and drooling for an appropriate time period. The ferret has also been used occasionally for safety evaluation studies [64].

LIVER AND GALL BLADDER

Experimental compounds may have significant effects on liver histology and function. In most cases abnormalities are found in chronic toxicology studies by observation of liver pathology or abnormal liver enzyme levels.

Classes of Compound with Potential Liability and Possible Mechanisms

Any experimental compound may affect bile secretion and gall bladder function. However, only those compounds with known structural or pharmacologic similarities to substances with activity on these functions need be tested.

Animal Models Available

The usual reason for collecting bile is to evaluate and quantify drug excretion in the bile. However, it also may be used for testing effects of compounds on bile flow, if effects are suspected. A rabbit or dog model must be used to evaluate activity directly on the gall bladder.

Effect on Bile Flow

Studies on bile flow are usually not part of safety pharmacology testing but done during acute, short-term or long-term safety evaluation. In some limited instances, effects on bile (content, volume) are required. Since the rat does not have a gall bladder it is not an appropriate species for the study of bile storage. Short-term effects on bile formation and transport to the small intestine may be done in rats. Drug excretion and bile content can be studied using acute or chronic bile duct cannulation to directly collect bile. If these studies are longer than a few hours, some mechanism for returning bile back into the small intestine must be used, since catheterization of the common bile duct prevents recycling through the entero-porto-hepato-biliary circulation. Steady-state conditions can be ensured by constant infusion of bile or bile acids into the stomach or duodenum. Urine and feces can also be collected to allow quantitative excretion of the compound(s) of interest.

Enderlin and Honohan [65] have published a method for long-term collection of bile, using an externalized cannula brought through an intrascapular incision. The cannula was protected by a 30-cm, 16-gauge stainless steel tube attached to the skin through the incision. The rat was housed in a cage at a level higher than the collection vial to aid in drainage of bile. A hole in the vial cap permitted the cannula to turn freely as the rat moved about the cage. This method allowed bile to be collected conveniently over long periods of time with minimal restraint of the animal.

In order to provide a steady-state condition, Enderlin and Honohan [66] describe a re-entrant cannula with a sampling arm that is inserted into the bile duct to allow intermittent collections of bile from unanesthetized, minimally restrained rats, for a period of 30 days or more. The flow rate of bile ($5.38 \text{ cm}^3/\text{h/kg}$) was higher than that previously reported when using simple or re-entrant cannulas. This higher flow rate may be a result of allowing a minimum of 10 days post-operative recovery period before starting collection of bile and of avoiding anesthesia and other stresses at the time of collection. Other methods may be found in [67–71].

Pancreatic Exocrine Secretion

Compounds suspected of having effects on volume and content of pancreatic juice should be tested.

Classes of Compound with Potential Liability and Possible Mechanisms

Many classes of drugs may affect the volume and content of pancreatic fluid. The measurement of insulin and/or glucagons levels is usually a function of longer-term safety evaluations and is not usually considered a gastrointestinal safety study. Although it is not usually performed, in some cases the information can be helpful in design of clinical trials and prevention of potential adverse effects.

Animal Models Available

Several methods for collection of pancreatic juice are available. Again, it is important that in experiments lasting more than 1 day that fluid and enzyme loss be replaced. A surgical procedure allows injection into the stomach and the duodenum by separate catheters, collection of the pancreatic juice during the experiments, recirculation of the pancreatic juice into the duodenum between experiments, and a normal circulation of bile in rats [72]. The following methods may also be used for collection of pancreatic juice in unanesthetized rats [73–75].

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CHAPTER 4

Gastrointestinal Tract Development and Its Importance in Toxicology

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GI TRACT DEVELOPMENT IN HUMANS

Prenatal Development

Digestion, absorption of nutrients, and elimination of waste products are the primary functions of the GI tract. Digestion and absorption of nutrients requires fully developed transport systems, functioning smooth muscle and enteric nervous systems, as well as digestive enzymes. Structural development of the GI tract occurs prior to the maturation of secretory function, immune function development, and normal motility throughout most of embryonic and fetal life (Doughty, 2004). [Table 4.1](#) provides an overview of the chronology of prenatal GI tract structural and functional development.

First Trimester

Embryonic Period

In early human embryonic development, most of the components of the GI tract arise from the endoderm germ cell layer during the second and third weeks of gestation. Areas of the GI tract appear to be regionally specified prior to the formation of the GI tube. During the fourth week, a tube forms the primitive gut and develops into three portions known as the foregut, midgut, and hindgut that extend the length of the embryo. This regional specification and orchestrated patterning of development is largely influenced by gradients of retinoic acid (Bayha et al., 2009). During this time, the oral cavity forms from the buccopharyngeal membrane arising from the mesoderm layer. The open tube contains amniotic fluid at this stage as well as at later stages when it is swallowed.

The earliest differentiated organ in the GI system is the liver, which is formed from the liver bud of the open GI tube beginning at week 3 of development ([Table 4.1](#)). Soon after, the pancreatic bud begins to form around weeks 3 to 4 (Podolsky et al., 2015). The midgut is a herniated umbilicus external to the abdomen during this stage of development.

During week 5, the spleen arises from the mesoderm, with the cells responsible for hematopoiesis derived from the yolk sac wall. An important step in early development is the rotation of this midgut to allow the GI tract to be in the appropriate position to develop mesentery derived from the mesoderm layer. During week 5, circular smooth muscle is detected in the esophagus, where neuroblasts are found dispersed among the muscle cells. As secretory cells differentiate and primitive structures form, muscle and neural cells develop into organized tissue during this time period as well (Stanger and Podolsky, 2015). Undifferentiated stem cells are present throughout the GI tract by week 5, giving rise in the small intestine and colon first to committed stem cells and then developing into four distinct lines of cells found in the intestines:

Table 4.1 Chronology of Prenatal GI Tract Structural and Functional Development

Gestational Week	Structure	Function
1		
2	Most of the components of the GI tract arise from the endoderm germ cell layer. Liver bud forms.	
3		
4		Tube forms the primitive gut—develops into foregut, midgut, hindgut. Oral cavity forms. Pancreatic bud forms.
5	Smooth muscle forms around esophagus. Neuroblasts present among esophageal cells. Spleen arises. Lumen of GI tract is occluded due to cellular growth.	
6	Rotation of primitive gut links vagus nerves to anterior and posterior stomach.	
7	Enteric nervous system development begins.	
8	Intestinal loops form. Appendix appears. Innervation of the small intestine begins.	Rudimentary glands develop mucous neck cells, parietal cells, and chief cells.
9	Lumen of GI tract re-opens following occlusion. Longitudinal muscle layer forms around myenteric plexus.	Secretory cells develop.
10	Differentiated endocrine cells present in the stomach and duodenum. Intercellular tight junctions begin to develop in gut epithelium.	Sucrase-isomaltase, maltase, and trehalase enzyme activity present in the small intestine. Secretory activity of substance P and neurokinin A occurs in stomach.
11	Intestinal crypt formation begins. Peyer's patches appear in gut.	Fetal swallowing is initiated. Gastric lipase activity appears. Dipeptidases activity begins in the small intestine microvilli.
12	Secretory acini and islets of Langerhans appear in pancreas.	Intestinal lactase activity begins; muc5B activity appears in pancreas,. Goblet cells begin producing mucus and paneth cells begin producing antibacterial proteins in intestinal barrier.
13	Neural cells appear in stomach; submucosal plexus formed. Intestinal villi formation complete.	Spleen begins generating red and white blood cells and leukocytes are transported to the gut.
14	Anatomical components of the stomach are distinguishable. Intrinsic factor and histamine-containing cells appear in stomach.	
15		

(Continued)

Table 4.1 (Continued) Chronology of Prenatal GI Tract Structural and Functional Development

Gestational Week	Structure	Function
16	B and T lymphocytes begin to appear in gut. Endocrine cells in intestine. Ductal and acinar cells rapidly developing in the pancreas. Peyer's patches aggregates of lymph nodules appear in small intestine. Muscularis mucosa is present throughout the small intestine to the colon. Neuronal density within the esophagus peaks.	Gastric lipase and microvilli dipeptidase activity functioning at adult level.
17		Proximal to distal gradients for activity of lactase and other glucosidases are evident.
18		Non-nutritive sucking activity begins.
19		
20		Active transport of glucose begins. Sucking behavior becomes more apparent.
21		
22		Bile secretion begins.
23		
24		Increased lactase activity.
25		Contraction pressure within intestines is functional.
26		Amino acid transporter activity and macromolecular transport across mucosal cells is evident. Peristaltic activity in GI tract begins.
27		
28		GI tract motility is developed, about 20%–25% than at birth.
29		
30		Enterokinase activity by pancreas begins; propulsive motility of GI tract begins.
31		
32		Pancreatic lipase activity is at 50% of the level at birth. Lower esophageal sphincter is functional.
33		Peristalsis of GI tract becomes effective.
34		Nutritive sucking and coordinated swallowing begin.
35		

- Columnar absorptive cells that become the major mucosal cells involved in absorption and local metabolism of nutrients as well as toxic contaminants
- Goblet cells responsible for mucus production
- Paneth's cells (secretory cells) found in the bases of crypts of Lieberkühn that contribute to mucosal barrier function by the apical release of granules containing a variety of antimicrobial products, including peptides termed cryptdins (crypt defensins)
- Enteroendocrine cells that appear by week 8 and are responsible in the duodenum for synthesis and release of hormones such as secretin and cholecystokinin (CCK) in response to nutrients and neural factors present in the upper GI tract

A sequence of cellular proliferation during weeks 5 and 6 results in the occlusion of the GI tract lumen that continues into week 7.

By week 6, the rotation of the primitive gut results in linking the left and right vagus nerves to the anterior and posterior areas of the stomach. The development of the enteric nervous system is initiated during week 7 when neural cells from the vagal and sacral regions of the neural crest migrate to the gut tube (Erdman and Pollack, 2001; Hamilton, 2000; Stanger and Podolsky, 2015).

By the end of the first 8 weeks, the intestinal loops return to the abdominal cavity and the appendix appears as the fetal development period begins. By week 8, enervation of the small intestine is initiated as synaptic protein and glial supporting tissue penetrate the outer layers of the developing circular musculature in the intestines. During the time that the midgut is herniated, the small intestine coils, the cecum forms, and by week 8 of gestation the descending colon is apparent (Hamilton, 2000).

Fetal Period

As the fetal period begins at week 9, cells responsible for secretory function become apparent. Within the primordial stomach, rudimentary glands develop mucous neck cells, then parietal and chief cells by week 8, with mRNA for mucl, 4, SB, and 6 detected in epithelial mucin in the stomach at that time. By the second trimester, the spleen is generating both red and white blood cells, thus initiating a mechanism for developing oxygen transport as well as leukocytes to the gut.

During week 9, the longitudinal muscle layer (inner layer within the mesenchyme) is formed along with the myenteric plexus, a network of nerve fibers between the outer longitudinal and middle circular muscle layers (Stanger and Podolsky, 2015). At this time, the intestinal lumen that had been occluded is re-established; first in the esophagus, then the stomach-duodenum, then the small intestine, and finally in the colon by week 10. This gradient in sequencing from proximal to distal sites occurs both in structural development and in subsequent secretory development.

By week 10, a fully differentiated spectrum of endocrine cell types is present in the stomach and duodenum. Sucrase-isomaltase, maltase, and trehalase enzymes are present in the small intestine at 60%–70% of adult levels. Substance P and neurokinin A can be detected in the stomach also at week 10. The gut epithelial barrier develops tight intercellular junctions beginning at week 10, contributing largely to immune function (Maheshwari and Zemlin, 2006). Intestinal epithelial

cells also have significant immune functions, including expression of HLA-I and HLA-DR, acting as non-professional antigen-presenting cells (and transferring antigen to lymphoid follicles and Peyer's patches below), and exhibiting receptors to facilitate immunoglobulin transport.

During week 11, gastric lipase appears, and dipeptidases are detected in the small intestine microvilli. Fetal swallowing is initiated during week 11. Amniotic fluid contains proteins, carbohydrates, triglycerides, and growth factors. The swallowing of amniotic fluid stimulates the secretion and development of enteric hormones that promote gut motility. During week 11 crypt formation in the GI tract begins, with villi formation completed by week 13 or 14 (Rubarth and Van Woudenberg, 2016). Paneth's cell defensins are considered early markers of crypt ontogeny and have been used to identify these periods of rapid villi development (Ayabe et al., 2000; Ouellette and Bevin, 2001; Stanger and Poldosky, 2015). Lymphoid tissue also appears early in fetal development at this stage with Peyer's patches and other organized lymphoid tissue, as well as scattered B and T lymphocytes appearing at 11–14 weeks (Maheshwari and Zemlin, 2006).

By week 12, intestinal lactase is measurable and pancreatic muc5B appears. Within the developing pancreas, secretory acini (responsible for pancreatic enzyme production and secretion) and islets of Langerhans (responsible for insulin, glucagon, and somatostatin production, among other hormones) appear also in week 12. Goblet cells and paneth cells of the epithelium become functional by week 12, with the goblet cells producing mucus and the paneth cells producing antibacterial proteins such as lysozyme and α -defensins (Maheshwari and Zemlin, 2006).

Second Trimester

The second trimester begins at week 13. By this phase in development, many of the major structures of the GI tract are generally in place, with specialized cell types beginning to appear and some limited functionality. As the second trimester continues through gestational week 28, more specialized cell types appear and organs begin to take shape. Specialized cells gain more functionality as they begin producing functional proteins and secretory factors.

Neural cells first appear in the stomach and the rectum by week 13, another example of the longitudinal gradient of development from a proximal to a distal direction. By the end of week 13, the submucous plexus is formed between the middle circular muscle layer and the submucosa, and mature ganglion cells are detected in the esophagus.

Endocrine cells in the GI tract are present beginning at week 14, and become active and functional around week 26. During week 14, the anatomical components of the stomach are distinguishable (curvatures, corpus, antrum, and pylorus) (Erdman and Pollack, 2001; Hamilton, 2000; Stanger and Podolsky, 2015). Intrinsic factor and histamine-containing cells are evident in the stomach by week 14, followed by the attainment of adult levels of gastric lipase by week 16.

Because pancreatic lipase, which is first detected during week 16, is not fully functional at adult levels until late into the first year of life (see [Table 4.2](#)), gastric

Table 4.2 Developmental Status of GI Tract Functions

Structure	Function	Developmental Status at Birth	Age When Fully Mature
Esophagus	Striated muscle in midesophagus	Same as adult	At birth
	Lower Esophageal Sphincter (LES) pressure	Partially functioning	3–6 weeks of age
	Swallowing	Poorly coordinated; rate = 450 mL/day in presence of mean amniotic fluid volume of 850 mL	
	Neuronal density	Adult level	
	Vagus nerves innervate striated muscle		2 years of age
Stomach/ Duodenum	Rate of DNA synthesis	Markedly higher than adult level, leading to significant mucosal growth	
	Gastrin G cells	Present in gastric antrum	
	Gastrin	High levels in neonatal plasma may act to increase crypt and villus density and parietal and ECL cells	
	Histamine response	Immature	1 year of age
	Somatostatin D cells	Appear in gastric mucosa	
	Gastric lipase activity		3 months of age
	Parietal cells secrete HCl	HCl <50% of adult values; pH ≈ 4–5	1 year of age, pH ≈ 1–2
	Pepsinogen secreted by chief cells	<50% of adult values	Pepsinogen at adult level by 1 year
	Intrinsic factor		Increases rapidly to adult value by day 10
	Motility	Not fully developed	
Small Intestine/ Colon	Enterochromaffin-like cells	Accelerate between 1–3 weeks of age	
	Bacterial colonization	Limited	Variable until 2–4 years
	Microvilli	Lengthening	
	Disaccharidases	Fully developed and functioning	At birth
	Glucose transporters	Fully developed and functioning	At birth
	Fructose transporter	Not present	
	Enterokinase	20% of adult level	
	Brush border and cytosolic peptidases	Fully developed and functioning	At birth

(Continued)

Table 4.2 (Continued) Developmental Status of GI Tract Functions

Structure	Function	Developmental Status at Birth	Age When Fully Mature
Small Intestine/Colon (Continued)	Amino acid transporters	Fully developed and functioning	At birth
	Pinocytosis	Higher rates in infant than in adult	
	Bile acid reabsorption	Passive in infant	By weaning
	Micelle formation	Poor due to low level of bile acids, pancreatic lipase	
	Passive and carrier-mediated absorption	Increased at birth above adult levels	
	Heavy metal (Cu, Fe, Zn, Pb) absorption	Efficiency > than in adults	Returns to normal at weaning
	Lactoferrin receptors	Present for milk at birth	
	Contractile activity and motility	Near adult levels	
	Fat-soluble vitamin absorption	Poor	Matures with weaning, normal fat absorption
	Folate absorption	Less than adult level	
	Vitamin B ₁₂ absorption	Not well absorbed in proximal ileum	
	Pancreatic lipase	10% of adult level	By weaning

lipase becomes the primary determinant of lipolytic activity in premature and newborn infants. Protein digestion at this gestational age is also developing; by week 16 they too are at adult levels. Leucine aminopeptidase activity in the *distal* small intestine is high at this gestational age (one of the few examples of a distal-to-proximal direction in development). Peyer’s patches begin as aggregates of HLA-DR⁺, CD4⁺ lymphoid cells, with B and T cells apparent by 16 weeks. Several weeks later, the B and T cells are in separate zones within the patches. Outside the organized lymphoid tissue, two kinds of B cells are found in the lamina propria: large mature, dividing cells that share characteristics of thymic cells and small cells that may develop locally (Maheshwari and Zemlin, 2006).

Proximal to distal gradients for activity of lactase and other glucosidases are evident by week 17. Non-nutritive fetal sucking begins around week 18 (Rubarth and Van Woudenberg, 2016). As the second trimester continues, ductal and acinar cells in the developing pancreas are undergoing rapid cell mitosis during week 19, and Peyer’s patches aggregates of lymph nodules emerge during week 20 in the small intestine (Stanger and Podolsky, 2015). By week 22 bile secretion is demonstrated, and active transport of glucose begins to occur. There are two kinds of intestinal T cells, intraepithelial lymphocytes and lamina propria lymphocytes. Lamina propria lymphocytes expand throughout fetal period, achieving a density similar to postnatal

levels by 19–27 weeks, while small numbers of intraepithelial lymphocytes expand rapidly after birth (Maheshwari and Zemlin, 2006).

While muscle cells and nerve cells are visible in the early gut in the early fetal stage, the muscularis mucosa becomes fully present throughout the small intestine to the colon by week 20. Neuronal density within the esophagus peaks by week 20 as well, resulting in swallowing rates of 13 mL/day at this gestational age, with sucking behavior apparent (Doughty, 2004).

By week 24, increased lactase activity is present indicating that induction by maternal lactose is not necessary. Contraction pressure within the intestines is at about 60% at week 25 compared to the level reached at term, indicating early ability to move intestinal contents along the developing gut. By the end of the second trimester (week 26), contraction is noted after electrical stimulation, indicating more coordinated activity. Motility measured at week 28 is about 20% to 25% that seen in a term infant (Doughty, 2004).

By the end of the second trimester (around week 26), six amino acid transporters for neutral and charged amino acid are found in mucosal cells and fetal macromolecular transport across the mucosal cell can be demonstrated—an important activity that aids in the digestion of proteins and lipophilic compounds present in amniotic fluid. At this time, the structural features of the GI tract are well developed (Erdman and Pollack, 2001; Hamilton, 2000; Stanger and Podolsky, 2015).

Third Trimester

By the start of the third trimester, at week 29, much of the major structural components of the GI tract and organs are developed and many specialized cell types have gained increased functional activity. During the third trimester, the GI system continues to gain more functionality and continued growth.

Early in the third-trimester trypsin activity is measurable in the small intestine, reflecting developing pancreatic function and the presence of active enterokinase. By week 30, enterokinase activity is at about 6% of adult levels. By week 32, pancreatic lipase activity is at 50% of the level at birth (although still low compared to adult levels) (Babyatsky and Podolsky, 2003).

During the third trimester, given that structural development is somewhat complete, improved function is noted. By week 32 the lower esophageal sphincter is functional, allowing more coordinated swallowing patterns to develop, although mature patterns of contractile activity and motility are not seen until near term, and coordinated contraction of smooth muscle in the colon and function of the internal and external anal sphincters (perhaps due to the proximal-distal gradient in development) are poorly developed before birth. Random peristaltic activity can be detected by the 26th week but effective peristalsis is usually not present until week 33, with nutritive sucking and coordinated swallowing not developing until week 34 (Doughty, 2004; Rubarth and Van Woudenberg, 2016). Lactase activity surges throughout the third trimester, while other disaccharidases reach adult levels prenatally (Stanger and Podolsky, 2015).

Postnatal Development

Many physiologists separate postnatal development into three time periods: birth and the first few days after birth (when colostrum is available), the suckling period, and the weaning period (Pacha, 2000). The weaning period marks the beginning of notable changes in digestive and transport functions that result in maturation of the GI system (Walthall et al., 2005). The timing of development of various GI tract functions after birth is described below and summarized in [Table 4.2](#). Also described below is the development of the GI tract microbiome, increasingly accepted as a critical component of GI system function.

General Postnatal Functional Development

Humans have been classified as precocial animals in that prior to birth, essential functions such as sight, hearing, hair, etc. are in place to allow for normal growth and development. This is in contrast to other animals such as rodents that have altricial characteristics: they become fully functioning during postnatal development rather than prenatally. Thus, functional maturation in humans occurs *in utero*, allowing viability in spite of prematurity prior to birth and even earlier for many functional attributes such as absorption, swallowing, etc. Thus, animal models used to evaluate potential human toxicity during pre- and postnatal periods must be chosen carefully to appropriately represent human age-dependent functional development.

At term, the small intestine is typically 250 to 300 cm (approximately one-half the length of the adult small bowel) and the large intestine and colon together are typically 30 to 40 cm in length (approximately one-fifth normal adult length) (Doughty, 2004). Thus the neonatal colon, mirroring the cephalocaudal pattern of development, is comparatively less functional than the small intestine. Solute and electrolyte absorption and bile reabsorption are not yet at mature stages.

Macromolecules given as tracers late *in utero*, either introduced into amniotic fluid or the intestinal lumen, are absorbed into mucosal cells in humans and a number of other species including monkeys, guinea pigs, and rats demonstrate a high rate of pinocytosis that is highly active in the first 10 days after birth and decreases substantially with weaning (Stanger and Podolsky, 2015). This provides a mechanism by which intact proteins are absorbed. This can be demonstrated in premature infants as well during the first few months of life. It has been suggested that the lack of a fully protective barrier during the first months of life may play a significant role in subsequent sensitivity to dietary and environmental proteins (Stanger and Podolsky, 2015).

At birth, while normal levels of many enzymes are present in humans so that digestion and subsequent absorption of nutrients can occur, a few important functions are not fully mature (see [Table 4.2](#)). A comparatively high rate of macromolecular transport important to protein and lipid digestion occurs due to pinocytosis during the first 2 weeks of life. This allows mucosal enzymes to assist in digestion of proteins and lipids when pancreatic proteolytic enzymes are not yet available in

adequate amounts. These are assisted by higher than normal levels of lysosomal proteases in enterocytes (e.g., cathepsins) that allow protein digestion.

In the early postnatal period, unhydrolyzed triglycerides are found in feces, a result of only passive bile acid reabsorption that continues until weaning. Bile acid concentrations are initially too low to allow for micelle formation, which requires pancreatic lipase as well as bile acids. During the first 4 to 6 weeks of life, intraluminal bile acids increase and absorptive mechanisms develop, leading to improved but not mature lipid absorption (Stanger and Podolsky, 2015).

Fewer chief cells are present in the neonatal stomach compared to the levels found in an adult stomach, with generally low levels of pepsin produced. The number of chief cells increases with age as the stomach increases in size and thickness. The mass of parietal cells (per unit area) at birth is two to three times greater than that of the adult stomach, but with thinner walls than found in that of an adult (Walthall et al., 2005). Acid output increases, as does pepsin production, resulting in improved digestion of proteins.

During the first week after birth the gut immune system undergoes many changes, including a reduction in mucosal permeability to macromolecules and bacteria (Maheshwari and Zemlin, 2006). Mucosal defense is aided shortly after birth by the secretory immunoglobulins, predominantly IgA. As the secretory immune system matures during the first year, there is a switch from monomeric IgA to polymeric sIgA.

Innate resistance to environmental and pathogenic microorganisms in the neonate is provided by Paneth's cells; considerable quantities of these cells are present in the small intestine, in all probability keeping bacterial levels low compared to levels present in the colon. Paneth's cells produce and secrete antimicrobial peptides known as defensins. Gram-negative and Gram-positive bacteria and their bacterial products have been shown to stimulate their secretion (Bourlioux et al., 2003; Porter et al., 2002).

In terms of muscle function, mature patterns of contractile activity and motility are not found until near term in humans, with coordinated contraction of colonic smooth muscle and anal sphincters responses poorly developed (Stanger and Podolsky, 2015). Sympathetic stimulation of the autonomic nerves reduces secretion and motility, while parasympathetic stimulation has been shown to increase peristalsis and secretion; these functions are immature at birth (Doughty, 2004).

By 1 month of age, pepsin activity is only at 18% of adult levels, and since the pH for maximum enzymatic activity of pepsin is ≈ 2 , while it may be present, it is not functioning at an optimal level due to the relatively high gastric pH, which is about 4.0 to 4.5 (Adkins and Lonnerdal, 2003). At the same time, the pancreas is still developing, with pancreatic function not attaining the adult stage until much later in infancy. Pancreatic enzymes such as trypsinogen that require intestinal enterokinase for cleavage to active forms are low at birth as well, limiting the luminal digestion of proteins; at birth, enterokinase activity is about 25% of that at 1 year of age (Adkins and Lonnerdal, 2003). It has been hypothesized that proteins in human milk may not be hydrolyzed in the small intestine, thus enabling them to exert a host-defense function beyond providing essential amino acids (Adkins and Lonnerdal, 2003).

Development and Functions of Intestinal Microbiota

Human beings are ecosystems consisting of a number of habitats, or niches, which house colonies of coevolved microorganisms that interact with each other and with the host. The human microbiota consists of all of the communities of human-associated microorganisms (bacteria, archaea, microeucaryotes, viruses, fungi) and the collection of their genes and gene products (RNA, proteins, metabolites) are referred to as the human microbiome. Some investigators include the environmental conditions in which these communities exist in the definition of the microbiome. The term *metagenome* is used to describe the collection of genomes from the members of a microbiota and the study of metagenomics seeks to understand the functions of a microbiota (National Academies of Science, Engineering, and Medicine, 2017).

Investigations into the nature and functions of the microbiome have accelerated greatly during the past decade, in large part due to the availability of methods that rely upon the analyses of marker genes amplified and sequenced from biological samples. The primary marker gene of the bacterial microbiome is 16S rRNA, and it is widely used to characterize bacterial microbiome communities. Although much of the study of the human microbiome is in its infancy, it is apparent that the human microbiome plays many important roles in physiology, metabolism, development, and health status.

The Human Microbiome Project (HMP), completed in 2012, has provided a vast reservoir of information on the microbiomes of the respiratory tract, skin, and gut (Human Microbiome Project Consortium, 2012). The HMP has revealed that the human microbiome exhibits a high degree of niche specificity. The microbiome of the oral cavity, for example, differs in composition and function from that of distal gut sites, and these are both quite distinct from skin and vaginal microbiomes. Indeed, variations along the length of the GI tract are also evident. Compositional variations exist across different age groups and are influenced by geography, diet, genetics, and health status (Human Microbiome Project Consortium, 2012).

The HMP and related research have provided evidence that humans are born with a simple “pioneer” population of microbes, and that this population expands and diversifies with age (Table 4.3). It appears that exposures to microorganisms occurring during gestation, birth, and early postnatal development may have lasting influences on health. The composition of microbial communities arising during these early life exposures varies among individuals because of factors such as gestational age, mode of delivery (vaginal versus cesarean section), type of feeding (breast versus bottle feeding), nutrition source (human milk versus formula), and medical interventions (Gritz and Bhandani, 2015; Hill et al., 2017).

One of the principal influences of the neonatal microbiome is on the development of host immunity, and these early life differences in the acquisition of microbiota may differentially affect subsequent health status related to immune system functioning. The gut microbiome also has significant influence on nutrient metabolism, and on the metabolism of drugs and environmental chemicals,

Table 4.3 Bacteria Commonly Found in Neonatal Gut^a

Phylum	Class	Genus Examples	Species Examples
Actinobacteria	Actinobacteria	<i>Bifidobacterium</i> <i>Atopobium</i> <i>Corynebacterium</i> <i>Propionobacterium</i>	<i>B. breve</i> , <i>B. bifidum</i> <i>spp.</i> <i>spp.</i> <i>spp.</i>
Bacteroidetes	Bacteroidetes	<i>Bacteriodes</i> <i>Prevotella</i>	<i>B. fragilis</i> , <i>B. vulgatus</i> <i>spp.</i>
Fermicotes	Bacilli	<i>Lactobacillus</i> <i>Enterococcus</i> <i>Clostridium</i> <i>Streptococcus</i> <i>Staphylococcus</i>	<i>L. acidophilus</i> , <i>L. delbrueckii</i> <i>E. faecalis</i> <i>C. perfringens</i> , <i>C. difficile</i> <i>S. mitis</i> <i>S. epidermidis</i>
	Clostridia	<i>Escherichia</i> <i>Klebsiella</i>	<i>E. coli</i> <i>K. oxytoca</i>
Proteobacteria	Gammaproteobacteria	<i>Enterobacter</i>	<i>spp.</i>

^a Composition is variable and is influenced by several factors, and diversifies over time (see text).

thus, as with host immunity, differential effects related to nutrition and chemical toxicities may be expected to arise because of differences in the composition and functions of microbiomes acquired in the early developmental period (Lynch and Pederson, 2016).

Dysbiosis, which is a general term used to describe any perturbation of the composition or function of a microbial community, may alter the normal roles of the microbiome and thereby affect health. The effects of chemicals on the composition and functions of the gut microbiome, and the health consequences of these effects, and the effects of the microbiome on the metabolism and uptake of chemicals, are becoming prime subjects for GI tract toxicology (National Academies of Science, Engineering, and Medicine, 2017).

Results from the HMP have given rise to much study of microbiome dysbiosis most especially in the gut microbiome, and increased risks of both local and systemic diseases. Thus, at least suggestive evidence exists to support associations between microbiome dysbiosis and obesity, Type II diabetes, colorectal cancer, ulcerative colitis, and Crohn’s disease (Lynch and Pederson, 2016). None of these associations is established as causal, and how the specific forms of dysbiosis and their causes relate to these disease states is poorly understood. These observations nevertheless raise testable hypotheses about the possible roles of chemicals (including pharmaceuticals) in microbiome dysbiosis, and the health consequences of those perturbations (National Academies of Science, Engineering, and Medicine, 2017).

Dysbiosis occurring during the developmental period may further alter microbiome functioning. In addition to altered nutrient metabolism and immune system development, dysbiosis occurring during the developmental period has been shown to alter nervous system development and has also been associated with asthma development in early childhood (Lynch and Pederson, 2016; National Academies of

Science, Engineering, and Medicine, 2017). As in the case of the other health effects noted earlier, the possible roles of chemical-induced dysbiosis in these disorders remains to be investigated.

HOW GI TRACT DEVELOPMENT AFFECTS CHEMICAL TOXICITY

Age-dependent changes in GI tract structure and function contribute significantly to age-dependent changes in toxicity. The principal causes of those changes are related to factors affecting the pharmacokinetic profiles of foreign compounds, although increased vulnerability of the neonatal GI tract to the direct action of some toxicants can also alter systemic responses. There are, of course, numerous age-dependent changes other than those occurring in the GI tract that affect both the pharmacokinetic and pharmacodynamic profiles of foreign compounds. Thus, the cumulative effects of age on both qualitative and quantitative manifestations of toxicity cannot be discerned solely from consideration of the status of the GI tract.

It is certainly the case, for example, that age-related changes in the GI tract that may serve to increase or decrease the toxicities of some substances may sometimes be countered by other (systemic) factors that are also age-related. Extreme caution is thus warranted when conclusions are drawn about the possible consequences for the whole organism of alterations in toxicity due to age-dependent effects of GI tract structure or function (Schumann et al., 1999).

Much of the information available about age-dependent changes in toxicity is necessarily based on experimental studies, and, as pointed out in [Chapter 10](#), there are several cross-species differences in GI tract structure and function that complicate interpretation of both rodent and non-rodent findings for purposes of assessing risks to humans. This situation holds even for well-developed models of mature animals and is compounded further when drawing inferences from findings in immature animals.

Because of the highly incomplete state of knowledge regarding risks of toxicity in immature humans and the similarly incomplete understanding of available animal models, much of the following is based on known age-dependent changes in the structure and function of the human GI tract that could affect toxicity; further development of experimental models is necessary to reveal the actual consequences of such changes. There are, however, notable examples of the influence of GI tract immaturity and these will be highlighted.

In addition to the effects of age-dependent alteration of GI tract structure and function on the toxic properties of foreign compounds, much concern focuses on the effects of such compounds on developmental processes, particularly on neurological development (National Research Council, 2001), but the possible consequences of *in utero* and neonatal exposures on the development of the GI tract (that may in part be related to neurodevelopmental toxicity) are also of interest. This topic will also be discussed in the following section.

Finally, as is now becoming clear, the gut microbiome can influence metabolism of chemicals and alter pharmacokinetic profiles. Chemical perturbations of

the microbiome may, in turn, alter the metabolic properties of the microbiome and perhaps affect the manifestations of chemical toxicity. Evolving knowledge of the microbiome is rapidly revealing new features of GI tract toxicity.

Alterations in Pharmacokinetic Profiles—Absorption

Absorption of nutrients and foreign compounds can be age-related. Absorption from the GI tract depends upon many factors including the type of diet and the timing of exposure relative to food intake, the pH levels of both GI tract contents and the foreign compound, the rate of passage of contents through the tract, and the surface area available for absorption. Certain disease states may also influence absorption. The diets of breast-fed and bottle-fed infants also affect nutrient and chemical absorption relative to adults. All these factors are altered in neonates, infants, and even young children relative to their status in adults. Their cumulative effects on any single drug or chemical are not readily predictable.

Gastric pH is higher in neonates relative to adults, and secretion of acid may not reach adult levels until early puberty (Havenaar et al., 2013; Makri et al., 2004). Although diet can alter gastric acidity, especially one high in milk and formula, pH generally remains high throughout the pre-school years (Makri et al., 2004). The absorption of hydrophilic compounds, particularly those that can undergo ionization, is most significantly affected by intragastric pH, because these compounds do not readily traverse the lipid membrane matrix when ionized. The relatively high pH of the immature GI tract as compared with adults will alter the predominant forms of weak acids and bases. A higher proportion of weak acids will exist in ionic form in the less acidic immature GI tract than in the mature tract, and thus will be less readily absorbed in infants than in adults. In contrast, absorption of basic compounds may be higher in infants. Absorption of lipid soluble compounds is generally little affected by pH.

GI tract transit times can alter absorption. Gastric transit time is generally longer in neonates and infants than in older children and adults (Makri et al., 2004), and is also delayed by diet, particularly one of high caloric density (Premji, 1998). These factors tend to increase the opportunities for absorption of foreign compounds in neonates and infants, especially for those chemicals absorbed primarily from the small intestine. Another factor contributing to potentially greater absorption in neonates and infants is the increased permeability and mucosal surface area relative to adults (Makri et al., 2004; Schumann et al., 1999; Sreedharan and Mehta, 2005). Immature GI tract motility and transit times may contribute to either reduction or increase in absorption or to changes in the time course of absorption, with prolonged gastric emptying delaying absorption of chemicals that are absorbed in the intestine, but enhancing absorption of chemicals absorbed in the stomach. Immature pancreatic function may reduce capacity to absorb lipids (Makri et al., 2004).

A range of enzymes involved in digestion and other biochemical functions including pepsin, lipase, α -amylase, β -glucuronidase, and UDP-glucuronyl transferase change in activity during the developmental period. Some of these changes may affect absorption of foreign compounds. Lower levels of bile acids present in

neonates may alter enterohepatic circulation of metabolites and otherwise affect absorption (de Belle et al., 1979; Watkins et al., 1973).

Altered drug absorption secondary to diseases such as infantile diarrhea, gastroenteritis, malabsorption syndrome, and inflammatory bowel disease has been reported for several pharmaceuticals (Maples et al., 2005). Reduced capacity for gastric emptying with consequent increased absorption has been reported for infants with congenital heart disease (Cavell, 1981).

The combined effects of these many factors influencing the GI tract absorption of chemicals are not readily predictable, but most tend to support increased absorption in neonates, infants, and perhaps young children relative to adults. Research on absorption in neonates and infants is focused on three broad categories of chemicals: nutrients (especially those that may be associated with deficiencies in infants), drugs that are frequently used to treat infant conditions or diseases, and xenobiotic chemicals that are likely to be transferred in breast milk or formula.

An example of a nutrient administered to infants is vitamin K (Shearer et al., 2012). Zinc deficiencies are also of concern (Kambe et al., 2015). Zinc absorption increases with the whey-to-casein ratio in milk (Ackland and Michalczyk, 2016). This ratio is higher in human milk compared with cow's milk or formulas. Thus, zinc absorption is higher in breast-fed infants than in adults or bottle-fed infants.

The pediatric pharmacology literature contains numerous examples of both increased and decreased drug bioavailability in neonates, infants, and children. Penicillin G, ampicillin, and nafcillin, for example, all display increased bioavailability in neonates relative to older children and adults (Maples et al., 2005). Reduced bioavailability and delayed absorption of phenobarbital, phenytoin, and acetaminophen have been reported in children (Maples et al., 2005). Increased bioavailability of drugs increases the risk of adverse side effects, while delayed absorption and reduced bioavailability may render them relatively ineffective. Recent studies are often focused on half-life and overall clearance, and do not always break out absorption factors (e.g., reviews of anesthetics by Allegaert et al., 2008, and of phenobarbital by Pacifici, 2016). Marsot et al. (2014) demonstrate that phenobarbital absolute bioavailability is close to 50% in neonates and infants as compared with a literature report of 95% for adults, a difference attributed to this drug being a weak acid affected by the higher gastric pH in infants.

Somani et al. (2016) provide a comparative population pharmacokinetic analysis of two classes of drugs using the biopharmaceutics classification system (BCS). Paracetamol and theophylline represented BCS class I drugs (high solubility, high permeability), while indomethacin and ibuprofen represented BCS class II drugs (low solubility, high permeability). For paracetamol, a one compartment model indicated that the rate at which the drug was absorbed in the GI tract reached adult levels within one week of birth. Increasing indomethacin and theophylline solubility by formulation changes changed the maturation rate to be more similar to the paracetamol model.

An improved approach to predicting oral absorption of drug formulations in neonates, infants, and toddlers was developed by Havenaar et al. (2013) who modified the TNO intestinal model (TIM). After conducting a literature search, they modified

key GI tract parameters in the model to represent the three age groups (birth to one month, 1 to 6 and 6 to 24 months), and found good predictive function when testing different dosage forms of frequently administered drugs. This predictive model is useful for examining the effects of drug manipulations such as crushing tablets or mixing with age-relevant foods. The drugs tested included paracetamol syrup and crushed tablets, crushed diclofenac tablets, and crushed enteric coated esomeprazole tablets. The model was validated by comparison with pediatric clinical data. With paracetamol, intestinal absorption was not dependent on age group, dosage form, or food matrix. In contrast, age-dependent differences in absorption were found for diclofenac and esomeprazole. The authors conclude this method will facilitate development of new pediatric dosage forms and inform labeling regarding dosage form manipulation.

Environmental toxicants of concern in neonates and infants differ for breast-fed and bottle-fed infants (Makri et al., 2004). For breast-fed infants, transfer of persistent organic chemicals stored in maternal lipids and metals stored in bone are of particular concern. Substantial doses of PCBs, dioxins, and lead may be transferred to infants during the duration of breast-feeding (Makri et al., 2004). Formula-fed infants may be exposed to a variety of waterborne chemicals, with nitrates being a prime example (Makri et al., 2004). Arsenic exposure may also be increased in formula-fed infants compared with breast-fed infants (Carignan et al., 2015).

Lead stands out as perhaps the most thoroughly investigated environmental toxicant. In both human and non-human primates, the age dependence of lead absorption rates is striking, with perhaps four to five times greater uptake in infants after oral exposure that has been observed in adults (Mahaffey et al., 2000). Much less clear is the age range over which absorption rates approach those of adults. By ages 6 to 7, and perhaps earlier, absorption rates become comparable to those of adults (Gulson et al., 2001).

More limited data on other heavy metals—mercury, cadmium, and nickel in particular—generally show the same trend observed for lead (Calabrese, 1986; Mahaffey et al., 2000). Studies of absorption are sometimes confounded by the fact that exposure of young children and adults to the same environmental sources and concentrations results in greater intake per unit of body weight. It is not always possible to separate and quantify the relative effects of increased intake and increased GI tract absorption (Mahaffey et al., 2000). Data on this issue compiled by Calabrese (1986) are presented in [Table 4.4](#).

Microbiome Influences on Chemical Metabolism and Pharmacokinetics

At birth, the microbiome is influenced by the maternal microbiome, delivery method, nutrition source, drug exposure, genetics, and environmental stressors (Cong et al., 2016). The structure of gut microbiome communities undergoes frequent changes until stabilization between the ages of 2 and 4 years (Tralau et al., 2015). Community fluctuations during this developmental period are influenced by nutrition (e.g., formula, breastmilk, and solid foods), exposure to antibiotics, and the developing

Table 4.4 Differences in GI Tract Absorption between Very Young and Adults

Chemical	Differences (Species)
Lead	5-fold (humans)
Cadmium	20-fold (guinea pigs) 4-fold (humans)
Strontium	9-fold (rats)
Plutonium	85- to 100-fold (rats)
Radium	24-fold (rats)

Source: Calabrese, E.J., *Age and Susceptibility to Toxic Substances*, John Wiley & Sons, New York, 1986.

immune system. The microbiome, in turn, influences the developing immune system as well as intestinal vascularization (Tralau et al., 2015). The importance of the role of the microbiome during the developmental period is described by Björkholm et al. (2009), who hypothesize that the influence of gut microbiota during development is necessary to affect homeostasis of the hypothalamic-pituitary-adrenal axis system.

It is well established that microbiota present in the gut (and at other sites) have a role in the metabolism of chemicals and in their uptake. Metabolism of arsenic, polycyclic aromatic hydrocarbons (PAHs), aromatic amines, and a number of other chemicals has been shown to occur in the gut microbiome. Direct transformation of chemicals by microbes in the gut appears to occur in much the same ways such transformations occur in liver cells. Microbiomes can also affect host liver metabolism. This would result in changes in the metabolites to which humans become exposed (National Academies of Science, Engineering, and Medicine, 2017).

From food additives (e.g., melamine, azo dyes, artificial sweeteners) to environmental toxicants (e.g., pesticides, PAHs, benzenes), the gut microbiome plays a varying role in both the toxification and detoxification of xenobiotics. The importance of these pathways observed in *in vitro* and animal models, and their relevance to the human GI tract, will require further study and is likely complicated by inter-individual differences and influences of diet and use of pharmaceuticals.

Metabolism of pharmaceuticals and other xenobiotics by the gut microbiome include reduction by azoreductases and nitroreductases, and hydrolysis by sulfatases, beta-glucuronidases, beta-lyases, and beta-flucuronidases (Claus et al., 2016; Spanogiannopoulos et al., 2016). Tralau et al. (2015) list pharmaceuticals metabolized in the gut, noting reactions with both positive and negative effects of the gut microbiome on drug efficacy as well as resulting side effects. In many cases, potential side effects associated with microbiome metabolism are unknown or not documented. In other cases, side effects are adverse to the host, as is the case with microbial-mediated azoreduction of sulfasalazine used in treatment of ulcerative colitis which has a positive influence on drug efficacy while also potentially contributing to microbe-induced anorexia, nausea, and skin rash.

Claus et al. (2016) provide an overview of gut-mediated metabolism of toxicants in both *in vitro* and *in vivo* models. Metabolic activation of PAHs resulting

in the generation of estrogenic metabolites is reported, as well as the regeneration of benzo(a)pyrene from hepatic conjugates. Nitrated PAHs are reported to be bioactivated by the gut microbiome, resulting in generation of DNA adducts (Hirayama et al., 2000), and by other metabolic pathways, resulting in the detoxification of mutagenic metabolites (Möller et al., 1988). Similarly, gut microbiome metabolism of PCBs has been shown to result in the formation of methyl sulfone metabolites which subsequently accumulate in adipose, liver, and lung tissues (Claus et al., 2016).

Mercury and arsenic are also metabolized in the gut. Methylmercury, for example, is demethylated by fecal bacteria to elemental mercury in rodent models, and *in vitro* models have shown that inorganic arsenic can be methylated to more toxic forms, such as monomethylarsonic and monomethylarsonous acids (National Academies of Science, Engineering, and Medicine, 2017). The translation of the *in vitro* findings to *in vivo* systems is yet to be verified (National Academies of Science, Engineering, and Medicine, 2017). Our understanding of microbial metabolism of pharmaceuticals is more advanced than that for environmental toxicants where the relative importance of their bioactivation and detoxification by the gut microbiome requires much further investigation.

Metabolic capacities vary throughout the developmental period, and the health risks of drugs and other chemicals may be quite different in the developmental period from those arising in adults, and this generalization is further strengthened when the metabolic capacities of the microbiome are considered. Immature microbiomes of neonates, infants, and children may play significant roles in the transformations of chemicals and drugs, with different health consequences than those associated with the microbiomes of adults.

Metabolism of foreign chemicals is not the only effect of the gut microbiome on chemical toxicity; there is evidence that host barrier integrity is affected by the microbiome, and uptake of chemicals from the GI tract may thereby be altered in different ways along the length of the GI tract (Sanogiannopoulos et al., 2016). For example, Cox et al. (2014) report an association between reduced ileal integrity and reduced microbial response to inflammation. Thus, compromises in tissue integrity as a result of exposure to xenobiotics or other factors may increase host susceptibility to exposures while also reducing the capacity of the microbiome to respond.

EXTRINSIC FACTORS AFFECTING DEVELOPMENT

Exposures to extrinsic factors such as xenobiotics or nutrients during critical periods of fetal and postnatal development are thought to have lasting or lifelong effects on subsequent structures or functions of organs (Hertzman, 1995) as well as impacting risk of chronic disease in later life (Barker et al., 2002). Relating perinatal exposure to long-term effects must take into account that factors that raise disease risk or promote good health accumulate gradually over the course of life both independently and in patterned ways (Ben-Shlomo and Kuh, 2002). Connecting early exposure or lack thereof to later health consequences, both positive and negative (life-course epidemiology), is not yet a well-researched model for establishing disease causation.

Nutrients and Dietary Components

The nutrient needs of the developing GI tract are met by nutrients present in the lumen derived from amniotic fluid and gut secretions during the fetal period, by milk and gut secretions postnatally, and by arterial blood flow during both periods of development. The comparatively high rate of mucosal cell turnover within the GI tract is functionally of great importance, allowing a means to protect an individual from adverse effects of constant exposure to potentially injurious substances present in the lumen, particularly in the colon, where mutagens and carcinogenic substances may be present.

Nutrients presented to the developing GI tract can act to regulate growth, functional patency, and cellular differentiation in three ways: by exerting direct effects on mucosal cells before joining the systemic circulation; by stimulating hormonal production in response to nutrient presence in the GI tract or post-absorption; and by affecting motor activity. Exposure to nutrients and other trophic factors in the diet has been shown to directly enhance villus height and crypt depth in the small intestine to a greater extent than in the ileal region. This has been shown to be a direct effect on the intestinal section itself, as transposition of ileal and jejunal intestinal loops results in increased villus size in the ileal segment (Erdman and Pollack, 2001; Perin et al., 1997; Sanderson, 1999; Stanger and Podolsky, 2015; Thiesen et al., 2000).

The presence of protein and fat in the duodenum and proximal small intestine results in the secretion of cholecystokinin (CCK) from specialized I cells in the duodenum. CCK is thought to play key roles in stimulating pancreatic secretion, gall-bladder contraction, and slowing down gastric motility, thus optimizing protein and fat digestion, which in neonates is still immature (Sidhu et al., 2000). The presence of some nutrient allergens (e.g., proteins from peanuts, eggs, etc.) in the developing GI tract was previously believed to have promoted IgE antigen response and food allergies. However, recent findings have suggested that exposure to these nutrients in the developing GI system actually mediates allergic response (Berin and Sampson, 2013; Lack, 2012).

Fetal under-nutrition has been linked with increased risk of disease in later life, specifically coronary heart disease, type 2 diabetes, stroke, and hypertension (Barker, 2004). Nutrient supply, including oxygen, has been shown to have more influence on fetal growth than genes (Harding, 2001). Smaller babies have fewer cells than would be proportionally expected in key organs such as the kidney and GI tract (Barker, 2004); this has been proposed as possibly a selective reduction resulting from diverting blood flow from the trunk to protect the brain. Along this line, it has been hypothesized that the tissue resistance seen to insulin in type 2 diabetes in adults may be a continuation of the fetal response noted in which glucose concentrations are maintained in the blood to provide adequate glucose to the brain at the expense of glucose transport into muscle, resulting in lower levels of muscle growth (Phillips, 1996).

Foreign Compounds

During prenatal development, the embryo is susceptible to chemical induction of malformations, while chemical effects during the fetal period are generally functional. Only 35 to 40 chemicals have been confirmed as human developmental toxicants (Rogers, 2013), and few are noted to affect the GI tract. Malformations of the GI tract may result from *in utero* exposures during critical period of fetal development. In many cases GI tract effects may represent one aspect of syndromes affecting multiple organ systems by common mechanisms. GI tract effects are not readily apparent at birth making epidemiological identification of the effects difficult. For example, thalidomide exposure between days 20 to 36 after fertilization has now been linked to a range of gastrointestinal effects, including anorectal stenosis, intestinal atresia, pyloric stenosis, and inguinal hernia (Vargesson, 2015); however, these effects are far less conspicuous than the pathognomonic limb malformations. Maternal smoking has been linked to various intestinal wall defects (including gastroschisis, omphalocele, anal atresia, and hernia) in a meta-analysis (Hackshaw et al., 2011), with increased odds ratios for GI tract effects also noted in a meta-analysis by Nicoletti et al. (2014). Animal studies provide additional evidence of GI tract malformations. Anorectal malformations have also been observed in rats following *in utero* exposure to ethylene thiourea (Hirai and Kuwahara, 1990); and similar malformations have been observed in mice following *in utero* exposure all-trans retinoic acid (Hashimoto et al., 2002). The mechanisms by which chemicals induce these effects are not yet fully understood (Rogers, 2013).

Greater susceptibility to GI tract toxicity in infants may be mediated by lack of well-developed metabolism and immune function, as well as higher dose rates per unit body weight. Increased vulnerabilities of infants and children are well documented in the case of various bacterial toxins that are causes of food and water-borne illnesses, where less-than-fully-developed immune status compromises the response.

Interactions between Chemicals and the Intestinal Microbiome

The role of the gut microbiome in modulating the toxicity of drugs and other chemicals is poorly understood. There is evidence that dysbiosis of the gut microbiome can be induced by certain chemicals (including some antibiotics), and some evidence relating to the health consequences of such an effect. The resilience (recovery) of the microbiome from such an effect is poorly studied. Stronger but still limited evidence exists to demonstrate a role of the microbiome in the metabolism and uptake of chemicals, with much yet to be learned. Animal models useful for studying such effects will be of limited relevance to humans unless greater success is had in developing humanized strains or with the use of relevant *in vitro* studies. The well-established population variability in gut microbiomes could be another important factor in assessing human risks related to toxicity outcomes mediated in some way by the microbiome.

Animal models are, of course, one of the principal tools of toxicology; however, differences in the timing of development of GI tract functions, especially just before and after birth, suggest that caution is needed in extrapolating from animal data to humans. This is especially apparent when considering murine and human microbiomes. When considering the many differences in GI tract environments, extrapolation of health effects findings in animals to humans when those effects are mediated by a chemical's action upon the microbiome, or by the microbiome's action on a chemical, can be especially problematic. While the ratio of mucosal tissue to body surface area is similar across species, distinct sections of the gut, which harbor microbiota, show considerable cross-species differences (National Academies of Science, Engineering, and Medicine, 2017). Many other such cross-species differences exist, including those relating to the development of immunocompetence. The CD4⁺ population in mice, for example, develops in the post-natal period, whereas human CD4⁺ populations are developed *in utero*. Studies of gut samples from humans and mice have generally shown minimal overlap of microbial genera (National Academies of Science, Engineering, and Medicine, 2017).

These observations of cross-species differences in microbiome compositions suggest that animal studies of chemically induced dysbiosis involving standard animal models may not reliably predict human outcomes. Although there is currently little direct evidence to support a significant role for chemical perturbations of the microbiome in the production of toxicity, the indirect evidence for such a role is significant, and points strongly to the need for additional study and the development of more clearly relevant experimental models.

One approach to dealing with this difficulty in the use of animal models involves efforts to “humanize” murine models. The process involves inoculation of germ-free animals with microbial species present in human feces. There has been some success in using such humanized (gnotobiotic) models in studying the effects of diet on the microbiome, but it seems clear that significant problems remain in creating murine microbiomes that are substantially similar to those of humans. Animal models, including gnotobiotic models, are playing key roles in furthering our understanding of chemical perturbations of the microbiome and their consequences, but extrapolation of results from such studies carries significant uncertainty (National Academies of Science, Engineering, and Medicine, 2017).

Yet research will expand, because it seems that the microbiome is quite possibly a critical target for the toxicology of the GI tract. How the developmental period may be particularly vulnerable to microbiome-mediated health effects is largely unknown, although there is suggestive evidence that such effects will be to some degree different from those occurring during later periods of life.

Antibiotics

That exposure to antibiotics can perturb the intestinal microbiome is not surprising. Numerous studies in both humans and animals have demonstrated various forms of antibiotic-induced dysbiosis (Vrieze et al., 2014). The primary effect seems to involve alteration of the relative numbers of certain microbial species rather than

alteration of the total numbers. These effects are often reported to be sex-specific and are influenced by age-related changes in development (Jin et al., 2017).

Candon et al. (2015), for example, reported that vancomycin treatment of mice increased levels of *Escherichia*, *Lactobacillus*, and *Sutterella* at the genera level, while decreasing levels of bacteria belonging to the order Clostridia and several families of bacteria. Total bacterial counts remained unchanged. These same investigators found, however, that co-administration of streptomycin, colestin, and ampicillin resulted in nearly complete elimination of gut microbiota in mice. These effects were associated with the appearance of interleukin-17 producing cells in the lamina propria of the ileum and colon, suggestive of a disruption of immune homeostasis (Candon et al., 2015). This is just one example of antibiotic-induced dysbiosis in the intestine leading to systemic effects.

Evidence is available that some microbiome-mediated effects of antibiotic treatment can lead to obesity and other metabolic disorders. The mechanisms for these processes are not yet fully understood (Cox et al., 2014; Jin et al., 2017). It also appears that the effects of some antibiotics on gut microbiota can persist for long periods of time (Jakobsson et al., 2010). For example, after cessation of pre-weaning exposure to low-dose penicillin, microbiome community composition in male and female mice recovered but the metabolic phenotypes resulting from the disruption persisted throughout adulthood (Cox et al., 2014). These lifelong metabolic changes following exposures during infancy resulted in increased adiposity as well as altered expression of genes related to intestinal immune function (Cox et al., 2014).

Some research on this topic has been devoted to the early life stages of gut microbiome development. Fouhy et al. (2012) found that the development of some bacterial species in infants treated with antibiotics seemed to be permanently altered with, in this case, unknown consequences. Vancomycin administration to mice in early life was reported to increase the risk of allergen asthma, apparently through increasing the abundance of Lactobacillaceae and Verrucomicrobiaceae; these increases were associated with decreased levels of regulatory T cells (Russell et al., 2012). Several studies of low level (sub-therapeutic) early life antibiotic treatments have reported increases in adipose mass in young mice (Cho et al., 2012, Cox et al., 2014). Some authors have suggested that low levels of antibiotics that have been detected in the environment and in foods derived from antibiotic-treated animals may be contributing to childhood obesity through microbiome-mediated metabolic effects, but there is at present no epidemiological evidence available to support such a hypothesis. It nonetheless remains clear that antibiotic-induced perturbations in the developing intestinal microbiome is an important subject for continued investigation.

Heavy Metals and Other Environmental Chemicals

Dysbiosis of the gut microbiome in animals has been induced by arsenic, cadmium, and lead (Patterson and Turnbaugh, 2014). Although it is clear that under certain conditions these metals can induce dysbiosis, including alterations in the metabolic functions of some of these organisms, the question of whether and how these effects result in adverse health effects in the host is only recently being addressed.

Wu et al. (2016) have reported, for example, that mice exposed perinatally to lead (32 mg/L in drinking water) for 40 weeks experienced a variety of alterations in gut microbiota, and exhibited increased body weights in adult male (but not female) mice. Gao et al. (2017) found that lead exposures in early life resulted in alterations in gut microbiome development over time, resulting in down-regulation of vitamin E, bile acids, and cholesterol synthesis and induced oxidative stress. Lukovac et al. (2014) reported that lead exposure virtually eliminated a bacterial species known to be necessary for development of the intestinal mucus layer.

Cadmium exposure in male mice at a concentration of 10 mg/L in drinking water for 10 weeks decreased the relative abundance of Firmicutes and Proteobacteria and increased that of Bacteroidetes. The authors found that these changes were associated with increased levels of serum lipopolysaccharides, hepatic inflammation, and energy metabolism regulation (Zhang et al., 2015). Early-life exposures in mice to low-dose cadmium (100 nM) resulted in increased adiposity later in life for male, but not female, mice (Ba et al., 2017).

Several studies have shown that metabolic profiles of gut microbiota can be substantially altered by arsenic exposure. For example, Lu et al. (2014) found that administration of 10 mg/L arsenic in drinking water for 4 weeks altered the abundance and diversity of gut microbiota in mice, as well as the regulation of gene and protein expression. The evidence is beginning to show that toxicities associated with these important environmental chemicals are mediated through their interactions with the intestinal microbiome.

Polychlorinated biphenyls (PCBs), chlorinated dibenzofurans, polybrominated diphenyl ethers, and PAHs have, under certain conditions, been shown to alter gut microbiota; PAHs have also been shown to be chemically altered (metabolized) by these organisms (Jin et al., 2017; Zhang et al., 2015). Following administration of high oral doses of 2,3,7,8-tetrachlorodibenzofuran in mice, Zhang et al. (2015) found that changes in microbiome community structure were mediated by activation of the aryl hydrocarbon receptor. The implications of these changes and relevance of these findings to concentrations encountered in the environment are yet to be elucidated.

Direct effects of a number of pesticides on the microbiome of the intestine have been reported. Although, most of these studies have not involved investigation of effects on host health. An *in vitro* GI tract model inoculated with adult human feces was used to evaluate effects of the organophosphate chlorpyrifos on the gut microbiome (Joly et al., 2013; Reygnier et al., 2016; Tirelli et al., 2007). In these studies, chlorpyrifos exposure contributed to increased membrane permeability and temporary changes in bacterial community structure and function. Gao et al. (2017) appear to provide the first report of a pesticide altering the structure and function of the gut microbiome in an animal model. In this novel study, an organophosphate concentration of 4 mg/L over 13 weeks resulted in sex-specific effects and suggested a potential for gut influences on neurotoxicity at doses below those associated with acetylcholinesterase inhibition.

It seems clear that investigations of the interactions of environmental chemicals of many types with the intestinal microbiome, and the health consequences of those interactions, will remain an important focus of GI tract toxicology.

Other Chemicals

Some chemicals found in consumer products and foods have been the subject of at least limited study (Javurek et al., 2016). Multiple studies report microbial changes resulting from exposure to aspartame, sucralose, and saccharin, in both animal and human models. Perhaps the most interesting of these studies is that reported by Suez et al. (2014). These investigators found that administration of saccharin, an artificial sweetener, to mice induced gut microbiome changes and glucose intolerance. Transplantation of the intestinal microbes from treated animals into germ-free mice also induced glucose intolerance. Mouse gut microbes cultured *in vitro* in the presence of saccharin induced intolerance when transferred to germ-free animals. This study provides useful information on experimental approaches to the investigation of chemical-microbiome interactions and their possible consequences for host health.

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CHAPTER 5

Gastrointestinal Tract as a Major Route of Pharmaceutical Administration

Robert W. Kapp, Jr.

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INTRODUCTION

The primary function of the gastrointestinal (GI) tract is the ingestion of foodstuffs which are subsequently broken down into basic nutrients for absorption for general sustenance. The daily diet of most individuals consists of about 2 to 3 pounds of food and 1½ liters of fluid containing as many as 100,000 different substances in amounts varying from about 1,000 grams to nanogram units. Of the 100,000 substances, only about 300 are classified as nutrients and of that number 45 are essential for the survival of the individual (Kapp, 2014). The remainder are passed through the system and eliminated primarily as feces. These ~300 nutrients provide energy to basic body processes and are critical to the building of new or the replacement of old components for various body structures. The structure of the small intestine is well suited to breakdown ingested foodstuffs because of the musculature, availability of digestive enzymes, secretory cells and immense surface area. Almost 80% of the contents of the GI tract entering the small intestine is absorbed before it enters the large intestine. Because the GI tract is designed to be effective at absorbing nutrients, it is also a target for the entry of a host of additional entities such as chemicals, bacteria and toxic substances. The GI tract has evolved to permit essential elements into the system and attempts to exclude substances which may be harmful or unnecessary. Evolutionary pressures created a series of barriers that are inherent in the mammalian digestive system that attempt to prevent the absorption of materials that are not recognized as essential substances. The most prominent barriers include the following (Kapp, 2014):

- Cell membranes of the intestinal mucosa which not only provide a physical barrier but produce enzymes can metabolize many substances which attempt to minimize the amount of toxicant that passes through the cell walls.
- Unstirred water layer which is an aqueous boundary located adjacent to the lumen of the GI tract. This layer acts as a diffusion barrier to various unwanted substances (Thomson and Wild, 2001).
- Acid microclimate is a layer closest to the mucosal surface where the proton concentration is higher than the bulk phase of the intestinal lumen. This layer is thought to be important for the absorption of weak acids such as folic acid and fatty acids; however, if the substance is too large or does not disintegrate or dissolve, it significantly decreases the amount that can be absorbed into the bloodstream (Thomson and Wild, 2001).
- The stomach pH is ~2 and not only degrades many unwanted chemical structures but destroys millions of unwanted bacteria that could be absorbed into the bloodstream.

In addition, if the substance is too large or does not disintegrate or dissolve, it significantly decreases the amount that can be absorbed into the bloodstream. The chemical structure may inhibit absorption if it is too large to move across cellular membranes or it does not dissolve or disintegrate if it may not be absorbed into the bloodstream. Additional barriers include the stomach with a pH of 3 to 5 which not only degrades many chemical structures but destroys millions of bacteria that could be absorbed into the bloodstream.

If a xenobiotic gets past these barriers, it is transported to the liver prior to entering the general circulation in a process referred to as the “first-pass” effect. These substances can be extensively metabolized in the liver prior to reaching the general blood circulation. Hence there are numerous hurdles for a foreign substance to overcome prior to its entrance into the general circulatory system. Among the many xenobiotics that are actively and passively prevented from absorbance are drugs.

Despite the myriad of hurdles drugs must overcome to gain entry into the body via ingestion, it remains the primary mode of drug administration. It is well established that the peroral route of drug administration is preferred by patients since it is—in most cases—the least stressful and most convenient mode of administration. Unfortunately, many drug formulations possess inherent solubility, absorption, distribution, and stability issues when exposed to the GI tract due to the barriers previously outlined. There are several major problems associated with drugs administered via oral ingestion (Kwan, 1997; Boddupalli et al., 2010; Verma et al., 2010):

- Inefficient—only part of the drug may be absorbed—poor permeability across the lumen wall
- First-pass effect—drugs absorbed orally are initially transported to the liver via the portal vein
- Irritation to gastric mucosa—nausea and vomiting
- Degradation of drugs by gastric acid and digestive juices
- Effect too slow for emergencies
- Unpleasant taste of some drugs
- Unable to use in unconscious patient
- Too rapid transit time across the ideal absorption site

However, since most drugs for oral administration are frequently less expensive to manufacture, are preferred by clinicians and patients alike and can be made into a variety of delivery formats including fast release tablets, slow-release tablets, capsules, enteric-coated, etc., there is considerable pressure on drug companies to produce drugs for oral administration.

Pharmaceutical companies search for effective drugs that possess a high potential to be absorbed in the GI tract, are well distributed in the circulatory system, and are not metabolized before it can reach the ultimately the receptor site. The primary considerations in drug development besides efficacy are physiochemical properties such as solubility, pKa, molecular size, hydrophobicity, hydrogen bonding capacity, particle size, complexation, and chemical stability. Physiological factors include membrane permeability, surface area of the site of adsorption, available transporters, variations in metabolism along the tract, amount and type of foodstuff

present, amount of water present, enterohepatic circulation engagement, intestinal and pancreatic secretions, transit time, and local pH (Burton et al., 2002; Alskör and Bergström, 2015).

The purpose of this chapter is to describe the issues concerning the gastrointestinal tract as a route of drug administration and the associated processes of drug absorption, bioavailability, and distribution. Since any xenobiotic or drug must overcome so many barriers to gain effective entry, the drug companies strive to manufacture oral medication that is successfully absorbed and ultimately transported to the receptor site.

FUNCTION AND STRUCTURE OF GASTROINTESTINAL TRACT

As previously noted, the primary function of the gastrointestinal tract is the absorption of nutrients and water from the surrounding environment for sustenance. The gastrointestinal tract is a specialized structure present in multi-cellular organisms that have the capability of providing nourishment to the organism to maintain life functions. The cell walls of the GI tract are composed of numerous tissue types which serve to break down foodstuff and fluid into absorbable components to create an environment for these components to be absorbed across the lumen into the circulatory system. The digestive system functions to modify the ingested luminal contents so that it can be absorbed from the lumen or “exterior” to the blood and/or lymph circulatory system or “interior” of the body (Kapp, 2014).

The GI tract is essentially a 25- to 30-foot series of hollow organs connected to form a pathway that begins at the mouth and proceeds through the pharynx, esophagus, stomach, small intestines, large intestines, rectum, and ends at the anus. Traditionally, the digestion system is organized into two divisions: (1) the GI tract or alimentary canal, which includes the mouth, pharynx, esophagus, stomach, small intestine, large intestine, rectum, and anus; and (2) accessory structures that include teeth, tongue, salivary glands, pharynx, liver, gallbladder, and pancreas. Although the lumen is found within the body, the contents within the lumen of the GI tract are considered to be external to the body and must traverse cell membranes to gain entry into the body’s circulatory system. The major organs of the digestive tract are displayed in [Figure 5.1](#).

Each portion of the GI tract is highly specialized and in conjunction with the accessory digestive organs that are connected through a series of ducts. These organs include the salivary glands, the pancreas, the liver, and the gall bladder. The GI tract in conjunction with the aforementioned digestive organs turn ingested foodstuffs into a thick semi-liquid mass of partially digested food, termed “chyme.” Chyme is further broken down and its nutrients are eventually absorbed in the small intestine for transport into the circulatory and lymphatic systems. [Table 5.1](#) summarizes the structure and primary functions of the various portions of the GI tract.

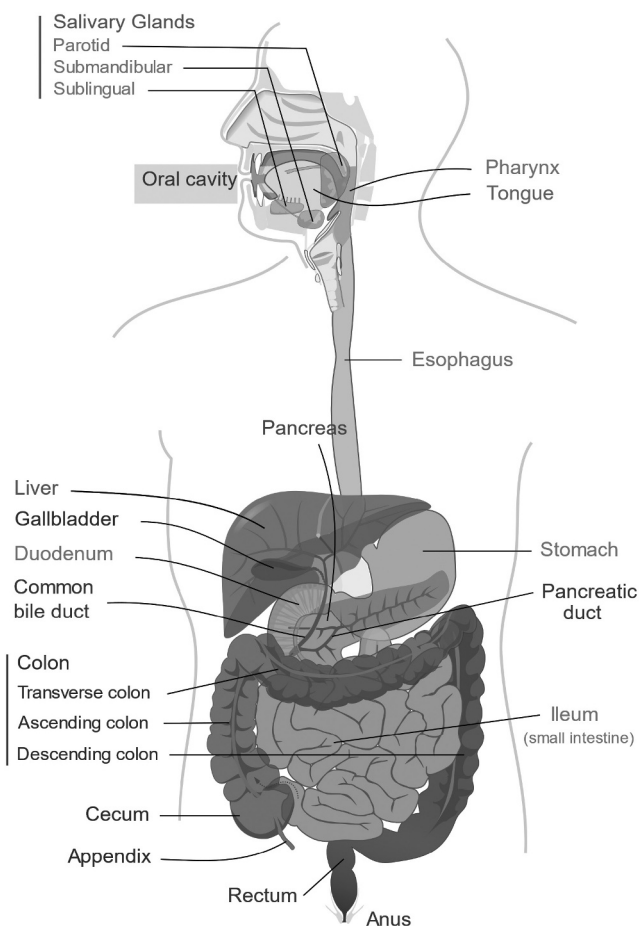


Figure 5.1 Major organs of the digestive tract. (Courtesy of Mariana Ruiz, LadyofHats.)

As noted in [Table 5.1](#), the GI tract presents various functions and conditions such as pH, transit time, and surface area. The oral cavity serves primarily as a place where foodstuffs or xenobiotics first enter in GI tract by tearing of smaller pieces of the food into smaller pieces by mastication. Saliva is added to the mix which includes the digestive enzymes amylase and ptyalin that breakdown starch into maltose and glucose. These substances form a bolus of material that rarely remains in the oral cavity long enough to be absorbed; however, some materials can be absorbed directly into the circulatory system bypassing the first pass metabolism of the liver via sublingual absorption. For example, nitroglycerin, used to treat certain heart

Table 5.1 Summary of Structure and Function of the GI Tract

Section of GI Tract	Functions	Secretory Glands	Accessory Organs	pH	Transit Time	Membrane	Vascularization	Surface Area	First-Pass Metabolism
Oral Cavity	Begin liquefaction mastication	Salivary Glands	Teeth; Tongue	~7	Brief	Thin	High	Limited	No
Esophagus	Conduit to stomach	Mucus secretion	Pharynx; Larynx	5–6	Brief	Thick	Normal	Limited	NA
Stomach	Reservoir mixing	Gastric acid glands	None	<3	30–45 minutes	Normal	Normal	Moderate	Yes
Small Intestine	Digestion and absorption	Intestinal juice; maltose; sucrose; lactase; amino-peptidase; dextrinase nuclease	Pancreas; Liver; Gall Bladder	6–8	3–4 hours	Normal	High	Very Large	Yes
Large Intestine	Bacterial digestion; water resorption	Alkaline mucus	None	5–7	24–30 hours	Normal	Normal	Moderate	Yes

Source: Kapp, R. W., Gastrointestinal tract as major route of pharmaceutical administration—Chapter 5, in Gad, S. C. (Ed.), *Toxicology of the Gastrointestinal Tract 1st Edition, Target Organ Toxicology Series*, CRC Press, Taylor & Francis Group, Boca Raton, FL, pp. 107–134, 2007; Bourne WAD, *Pharmacokinetics and Biopharmaceutics PHAR 7632/7633 & 4634*, College of Pharmacy, University of Oklahoma Health Sciences Center, Oklahoma City, OK. <http://www.boomer.org/>, 2017.

conditions, is commonly administered in this fashion since the material can quickly get into the bloodstream. As the bolus passes from the oral cavity, it enters the esophagus in route to the stomach. The esophagus is thick and muscular, and the material quickly passes through this section with little to no absorption occurring in transit. The bolus is subsequently delivered to the stomach which has a pH of 3 or less and a capacity of about one liter. The highly acidic stomach contents not only destroy the bacteria that enter with the foreign materials but activate pepsinogen in an initial step in the breakdown of proteins. The stomach fluid also contains mucus which provides the stomach lining protection from the high acidity of the contents. The stomach serves to mix the contents into a pulpy acid fluid consisting of gastric fluids and partly digested foodstuff which is now defined as chyme for its continuation into the next part of the digestive tract (Barr and Almond, 2015). From the stomach, the chyme passes into the first section of the small intestine identified as the duodenum where the pH varies from about 6 to 6.5 and the majority of the absorption into the circulatory system occurs. In humans, the duodenum is a hollow jointed tube about 25–38 cm (10–15 in.) long connecting the stomach to the jejunum which is the next section of the small intestine. Numerous digestive or “intestinal juices” are added in the duodenum along with pancreatic and bile secretions which are critical to the digestive process. The surface area of the duodenum is quite large—providing an extensive area for food and xenobiotic substances to be absorbed. The cell wall of this portion of the small intestine has millions of finger-like projections called villi which can be found on projections called the brush borders which are designed to provide a vast area for nutrient absorption. Each villus is embedded into the connective tissue of the wall of the small intestine with an arteriole, a venule, a capillary network, and a lymphatic vessel (lacteal), which allows nutrients to pass directly through the capillary wall directly into the lymphatic and cardiovascular systems. The jejunum and ileum lie beyond the duodenum and—while they certainly compose the longest part of the small intestine—they provide only a fraction of the absorptive surface of the duodenum. The jejunum possesses more absorptive surface area than the ileum which possesses the least area (Gabe, 2015). The pH begins to become more basic as the distance from the stomach increases. The total time in transit through the entire small intestine is about 3 to 4 hours. The final section of the GI tract is the large intestine or colon. This section is the primary site of water and electrolyte resorption. The pH of the large intestine ranges from 5 to 7. The blood supply to the rectum is not transported to the liver for the first pass metabolism. It is similar to the sublingual area in that anything absorbed there such as suppositories and enemas goes directly into systemic circulation. The transit time through the colon comparatively extensive and can be over 24 hours (Lunniss, 2015).

PHARMACOKINETICS

Pharmacokinetics (PK) generally refers to the mathematics of movement of chemical substances through the body including the way in which the body's physiology affects the substances over time. A drug PK profile determines the onset,

duration, and intensity of a drug's effect and is dependent upon such factors as the patient's sex, age, genetic makeup, renal function, etc. as well as on the drug's chemical properties. This includes how the body affects a specific substance after administration through absorption and distribution, as well as the metabolic changes of the substance in the body due to various influences such as cytochrome P450 or glucuronosyltransferase enzymes, and the effects and routes of excretion of the metabolites of the substance. PK properties of substances are affected by the route of administration and the dose of administered of the substance. The composition of the substance affects the absorption, the specificity of where that substance may be transported, the degree of metabolism, and the rapidity of excretion—also referred to as ADME. ADME is an acronym for Absorption, Distribution, Metabolism, and Excretion. These functions describe the effectiveness of a pharmaceutical compound. Each of these four criteria are all critical with respect to the success of the compound as a drug. The biological, physiological, and physicochemical factors which influence the transfer processes of drugs in the body also influence the rate and extent of ADME of those drugs in the body. In many cases, pharmacological action, as well as toxicological action, is related to plasma concentration of drugs (Porter, 2018). Consequently, through the study of pharmacokinetics, the pharmacist can individualize therapy for the patient. Further examination into PK requires examination of bioavailability as well as the specifics of ADME.

Bioavailability

Bioavailability refers to the constellation of activities associated with the processes necessary for the entrance of an active xenobiotic substance into the systemic circulation and its eventual occurrence at a designated receptor site where it can have an effect. Hence, “bioavailability” is a pharmacokinetic term that describes the rate and extent to which a therapeutically active drug is absorbed from a drug matrix and becomes available at the receptor site of drug action (Banakar and Makoid, 1997; Duan, 2016).

Oral absorptive mechanisms are a complex series of processes that are dependent upon several factors including chemical properties of the substance as well as physiological factors inherent within the lumen of the GI tract. Orally administered drugs must pass through the wall of the GI tract and into the portal circulation to the liver. This results in first-pass metabolism (metabolism that occurs before a drug reaches systemic circulation) which occurs as an innate protective mechanism in the liver. However, in the case of oral administration of pharmaceuticals, it hinders the absorption of the substance. Thus, many drugs may be metabolized before the proper plasma concentrations are reached. Oral administration of drugs that have low solubility and cannot readily penetrate the membrane of the GI tract wall generally also have low bioavailability (Savjani et al., 2012). The general health of the patient as well as specific chemical reactions of the drug is key factors in successful absorption. Chemical reactions include but are not limited to hydrolysis by gastric acid and/or digestive enzymes, formation of chemical complexes, conjugation within the intestinal wall, metabolic catabolism by GI tract flora, and possible adsorption to other xenobiotics.

Drug concentrations usually cannot be easily measured at the receptor site; therefore, most bioavailability studies involve the determination of drug concentration in the blood or urine. This is based upon the premise that the drug at the site of action is in equilibrium with the drug in the circulatory system. It is postulated that one can obtain an indirect measure of drug response by monitoring drug levels in the blood and/or urine. The bioavailability of a drug product affects the onset, intensity, and duration of therapeutic response of the drug and subsequently can determine the therapeutic efficacy of that substance. Thus, oral bioavailability is defined as the extent of original drug appearing in blood after oral administration (Gamlen and Cummings, 2015).

Bioavailability is often assessed by determining the area under the plasma concentration–time curve (AUC). The most reliable measure of a drug's bioavailability is AUC. The AUC is directly proportional to the total amount of unchanged drug that reaches systemic circulation. The AUC represents the total drug exposure over time. Therefore, assuming linear pharmacodynamics with elimination rate constant K , one can show that AUC is proportional to the total amount of drug absorbed by the body. The proportionality constant is $1/K$. AUC is useful for knowing the average concentration over a time interval, AUC/t . Drug products may be considered bioequivalent in extent and rate of absorption if their plasma concentration curves are essentially superimposable (Rolan and Molnár, 2015).

The bioavailability of oral administration (F_{oral}) is generally expressed by comparing the amount of drug in the circulatory system post oral administration as compared to that amount of drug in the circulatory system post intravenous administration. f_{absorbed} is the fraction of the intact drug taken into the intestinal tissue. $f_{\text{gut wall}}$ is the fraction of drug that remains after intestinal metabolism and enters the portal vein. f_{hepatic} is the fraction of drug that remains following first-pass intestinal and hepatic metabolism (Pond and Tozer, 1984). The mathematical relationship of the bioavailability of a drug is expressed in the following equation:

$$F_{\text{oral}} = (f_{\text{absorbed}})(f_{\text{gut wall}})(f_{\text{hepatic}})$$

Thus, the degree of bioavailability is comprised of individual fractions that survive the barriers encountered by the substance during its passage from the lumen of the GI tract to the point of sampling. In the above equation, f_{absorbed} is the fraction of substance absorbed (net transport of unchanged substance into the absorptive cells of the GI tract), $f_{\text{gut wall}}$ is the fraction that is not metabolized in a single passage through the gut wall, and f_{hepatic} is the fraction that is not extracted during the first passage through the liver. In most cases one is concerned with the extent of absorption of drug (i.e., the fraction of the dose that reaches the bloodstream), since this represents the “effective dose” of a drug. In general, there are numerous items that can and do influence the bioavailability of a drug. There are many barriers that can prevent a drug from reaching the ultimate receptor sites to be effective.

Christopher Lipinski's seminal manuscript (Lipinski et al., 1997) about the “rule-of-five” (RO5) identifies a series of features commonly found in orally active drugs.

The guidelines have been unofficially adopted by the pharmaceutical industry in an effort to predict which chemicals in early drug development might have the best possibility of passive transcellular transport and permeability (Pollastri, 2010; Celerino, 2013; Simpson and Scott, 2017). The “rule of five” is so called because most of the features start with the number five or are multiples of five. Variations to the rule have flourished to find more suitable drugs (Rondeau and Schreuder, 2015; Leeson, 2016; Singh, 2016; Goodwin et al., 2017). However, the basis of the “rule of five” is that most of the features start with the number five or are multiples of five and the concept remains mostly unchanged from the original publication (Lipinski et al., 1997):

- Molecular weight < 500
- Low lipophilicity (expressed as cLogP < 5)
- Not more than 5 hydrogen bond donors
- Not more than 10 hydrogen bond acceptors

ABSORPTION

Absorption is defined as the transfer of a substance from its site of entry to the circulatory or lymphatic system. The rate and efficiency of absorption of any substance administered orally are dependent upon numerous factors such as:

- Patient age, gender, disease state
- Disintegration and drug dissolution: foodstuffs, GI tract pH, pKa, solubility, lipophilicity, partial size, rate of peristalsis
- GI tract permeation: food–drug interactions, uptake and efflux transporters
- Drug to portal vein: hepatic metabolism such as CYP 450 enzymes, drug–drug interactions, protein binding

The above factors affecting the eventuation of the drug into the circulatory system is detailed in numerous publications including Gavhane and Yadav (2012), Doogue and Polasek (2013), Yang et al. (2017), Narang et al. (2017), Shekhawat and Pokharkar (2017), Porter (2018). The Biopharmaceutics Classification System (BCS) was promulgated as a science-based approach to assist in classifying drugs based upon their solubility and permeability and is currently in use at the USFDA as a biowaiver criterion (Koziolek et al., 2016; USFDA, 2017). Its use in drug discovery has been established to allow predicting the *in vivo* pharmacokinetic performance of drug products (Chavda et al., 2010; Arrunátegui et al., 2015; Sinai Kunde et al., 2015).

As a substance passes through the GI tract, it can be absorbed by one and more of several mechanisms. Many of existing nutrient absorption pathways in the GI tract are also used by foreign substances—such as drugs—to enter the body. Generally, if a drug is an organic acid or base, it most likely will be absorbed via simple diffusion. Based on the structure and chemistry of these substances, one can estimate that an organic acid exists primarily in lipid-soluble form in the stomach and in the ionized form in the small intestines. Hence, an organic acid is absorbed more readily in the acid stomach than in the neutral small intestine. Organic bases, on the other hand,

are absorbed more readily in the more neutral pH small intestine rather than in the acidic stomach. However, since the pH is about neutral in the small intestine, both weak acids and weak bases are non-ionized and readily absorbed by passive diffusion. There are significant exceptions to this due to the large surface area of the small intestine, varying blood flow rate and law of mass action maintaining a gradient in the compartments.

In addition to passive transport mechanisms, there are a variety of active transport and facilitated mechanisms that transport nutrients including monosaccharides, amino acids, and minerals into the mucosal epithelial cells. A xenobiotic exit transport mechanism has been identified which is called the multi-drug-resistant (mdr) protein or p-glycoprotein. P-gp is a member of the adenosine triphosphate (ATP)-binding cassette (ABC) family that is encoded by the human ABCB1 gene (ABC, subfamily B), also called MDR1. P-gp functions as a transmembrane efflux pump, thereby moving drugs from the intracellular to the extracellular domain; it may also interact with drug molecules trapped within the cell membrane lipid bilayer (Annese et al., 2006). This mechanism results in moving certain xenobiotics out of the cell which, in turn, acts to decrease the net amount of GI absorption for some drugs and toxicants. The multi-resistant drug proteins transport systems (mrp) have been shown to transport glucuronides and glutathione metabolites out of cells as well (Choi, 2005; Lehman-McKeeman, 2015). ABC transporters are widespread in all forms of life and are characterized by two nucleotide-binding domains (NBD) and two transmembrane domains (TMDs). ATP hydrolysis on the NBD drives conformational changes in the TMD, resulting in alternating access from inside and outside of the cell for unidirectional transport across the lipid bilayer. There are 48 ABC transporters in humans and many have been shown to be responsible for or involved in disease states, including cystic fibrosis, Tangier disease, adrenoleukodystrophy, and cancer (Wilkens, 2015). There are two other ABC transporters, which have been shown to participate in the multidrug resistance of tumors: the multidrug resistance protein 1 (MRP1) and the mitoxantrone resistance protein (MXR). Other human ABC proteins capable of actively transporting various compounds out of the cells may also be involved in selected cases of multidrug resistance. These include homologues of MRP1, MRP2–MRP5. MRP2 and MRP3 have been shown to be active in organic conjugate transport in various tissues, while MRP4 and MRP5 appear to have special functions as nucleoside transporters. MDR1 and MRP1 can recognize and transport a large variety of hydrophobic drugs, and MRP1 can also extrude anionic drugs or drug conjugates (Limtrakul et al., 2007). There are several suggested methods to prevent the expression or function of multidrug transporters; however, pharmacological modulation appears to be the first choice at present. The iron transport mechanism also absorbs xenobiotics such as thallium while manganese and cobalt both utilize and also compete for access to the iron transport system; the calcium transport mechanism also absorbs lead; the pyrimidine transport system also absorbs 5-fluorouracil. While these xenobiotics are actively transported into the mucosal epithelial cells of the GI tract, the vast majority of toxicants and drugs enter by simple passive diffusion. Low molecular weight lipid-insoluble compounds are absorbed through aqueous membrane pores at the tight junctions in the membrane

via passive diffusion. Another factor in the absorption of xenobiotics is that particulate drugs can be absorbed via the vesicular transport—either by pinocytosis or phagocytosis (Lehman-McKeeman, 2015).

Physical factors can markedly affect absorption include a drug's design such as whether or not it is a tablet, capsule, controlled-release formulations, etc. These factors are summarized in [Table 5.2](#).

Additional factors affecting absorption such as chemical properties of a drug are summarized in [Table 5.3](#).

Table 5.2 Critical Physical Factors Affecting Drug Absorption

Dosage Form	Variable	Effect
Solutions such as syrups and elixirs	Already in available aqueous form	Readily absorbable
Suspensions	In an aqueous form however, suspended particles must be disintegrated	Less readily absorbable than solutions
Capsules	Capsules are the most readily disintegratable form of solid dosages since the drug inside the capsule is easily dissolved	Less readily absorbable than suspensions
Uncoated tablets	Tablets are solids compressed into small pills which must be disintegrated before they can be dissolved	Less readily absorbable than capsules since the tablet must be disintegrated
Enteric-coated tablets	These tablets have coatings which are more difficult to disintegrate than uncoated tablets above	Less readily absorbable than un-coated tablets since the coating is specifically made not to disintegrate upon administration.
Controlled-released capsules	These capsules are designed to slowly dissolve over time, so the absorption is sequential and constant	Less readily absorbable than enteric-coated tablets
Controlled-release tablets	These tablets are designed to dissolve over a longer period of time for sequential and constant absorption	Generally, less readily absorbable than controlled-release capsules

Source: Kapp, R.W., Gastrointestinal tract as major route of pharmaceutical administration—[Chapter 5](#), in Gad, S.C. (Ed.), *Toxicology of the Gastrointestinal Tract 1st Edition, Target Organ Toxicology Series*, CRC Press, Taylor & Francis Group, Boca Raton, FL, pp. 107–134, 2007.

Table 5.3 Critical Chemical Factors Affecting Drug Absorption

Chemical Property of Drug	Variable	Effect
Particle/Molecular size	Degree of disintegration	Larger size results in less absorption
Hydrogen bonding	Degree of hydrogen bonding	Increases transit time and degree of absorption (Whitehead et al., 2004)
Partition coefficient	Pathway of absorption	Paracellular or transcellular route or not transported (Kulkarni et al., 2002)
Hydrophilicity	Tight junctions between epithelial cells	Prevents drug from passing through the cellular membrane tight junctions
Lipophilicity	Adipose tissue	Drug becomes trapped in adipose tissue
Ion trapping	Localization at non-receptor sites	Weak acid/or base drugs become trapped at sites such as the kidney and cannot reach the receptor site
Crystalline structure	Degree of hydration of drug active ingredient	Availability at membrane wall
Stability	Intact active ingredient intact long enough to be absorbed	Less stable drugs have decreased absorption
Low pH	Stomach	Degradation of active ingredients
Ion trapping	Non-receptor sites	Weak acid/or base drugs becomes trapped in sites such as the kidney and cannot reach the receptor site

Source: Kapp, R.W., Gastrointestinal tract as major route of pharmaceutical administration—[Chapter 5](#), In Gad, S.C. (Ed.), *Toxicology of the Gastrointestinal Tract 1st ed.*, *Target Organ Toxicology Series*, CRC Press, Taylor & Francis Group, Boca Raton, FL, pp. 107–134, 2007.

Physiological factors affecting absorption such as characteristics of the individual patient are summarized in [Table 5.4](#).

Lastly, there are additional factors affecting absorption of a substance such as drug–drug interactions as shown in [Table 5.5](#).

Table 5.4 Critical Physiological Factors Affecting Drug Absorption

Physiological Properties	Variable	Effect
Intestinal contents	Amount of food or other chemicals already present with the substance to be absorbed	Binding or complexation can occur affecting the amount of drug available for absorption (Galia et al., 1998)
Gastrointestinal microorganisms	Amount and location of microorganisms	Metabolism of active ingredients to inactive forms
Regional variations in pH of digestive fluids	Fluid volume and secretion rate of intestinal enzymes	Changes in degradation rate and changes in partition coefficients can increase or decrease absorption rates (Fallingborg et al., 1989)
Regional variations in absorptive capabilities of the digestive tract	Duodenum is very absorptive while the jejunum and ileum are less absorptive	Certain areas of the GI tract are more prone to enhance absorption than others
Intestinal motility	Transit can increase or decrease	In most cases, the slower the transit rate the more absorption can occur (Pang, 2003)
Gastric emptying	Gastric emptying rate can increase with stress, increased pH or can decrease with increased viscosity of GI tract content, exercise, eating fatty acids or carbohydrates, decreased pH	The less time the substance has in the GI tract, generally the less absorption occurs (Pang, 2003)
Intestinal metabolism	Metabolism occurring in the cell walls of the GI tract	Increases the first pass metabolism of the liver which can deactivate or activate a drug
Intestinal blood flow	Blood flow varies with stomach contents	Increased blood flow decreases the transit time through the gut and liver lessening the potential for first-pass metabolism
Aqueous boundary layer or “unstirred water layer”	Water/mucus layer surrounding the villi of the small intestine	Substances must diffuse across this layer to gain access to the mucosal cell membranes
Age, weight, sex	Each of these present biological variables that can affect absorption rates	Variable effects are noted in absorption rates with each of these variables
Pathology	Various disease state may affect the rate of drug absorption	Variable effects are noted in the absorption rates with many disease states in man

Source: Kapp, R.W., Gastrointestinal tract as major route of pharmaceutical administration—[Chapter 5](#), in Gad, S.C. (Ed.), *Toxicology of the Gastrointestinal Tract 1st ed.*, Target Organ Toxicology Series, CRC Press, Taylor & Francis Group, Boca Raton, FL, pp. 107–134, 2007.

Table 5.5 Drug–Drug Interactions

Item	Variable	Effect
pH	Acidic or basic—indirect effect	pH of one drug can affect a change the absorption of another by changing drug ionization state
Gastric motility	Changes in the rate of transit—indirect effect	One drug can speed up or slow the transit rate in the GI tract which can affect the absorption rate of the second drug
Drug-induced malabsorption	Interference with mucosal function—indirect effect	One drug can change the properties of the intestinal mucosa which can affect the absorption rate of a second drug
Chelation	Complexing with another drug—direct effect	One drug can chemically bind with another reducing the absorption of both
Exchange resin binding	Adsorption of resin of one drug with active moiety of a second drug—direct effect	One drug can bind with components of another reducing the absorption of both

Source: Kapp, R.W., Gastrointestinal tract as major route of pharmaceutical administration—Chapter 5, in Gad, S.C. (Ed.), *Toxicology of the Gastrointestinal Tract 1st ed.*, Target Organ Toxicology Series, CRC Press, Taylor & Francis Group, Boca Raton, FL, pp. 107–134, 2007.

On balance, the rate of absorption is primarily determined by the solubility of the active ingredient and the permeability of the cell wall to that solute. Generally, a drug consists of the active ingredient as well as inactive excipients such as diluents, disintegrants, stabilizers, and lubricants which are compressed together in varying degrees to enhance absorption and maintain stability. If the drug is administered in solution, it is already in a form that is theoretically absorbable. However, the solution must not degrade significantly considering the acidic pH and enzymatic secretions of the GI tract to be effectively absorbed. If the drug is administered in a solid form such as a tablet or capsule, additional steps are necessary in the process to facilitate absorption: (1) disintegration of the substance, (2) dissolution of the substance in proximity of the critical absorption site, and (3) transfer of the substance across the lumen of the GI tract into the circulatory or lymphatic system.

Disintegration increases the surface area of the substance which enhances the probability of the solid dissolving into the solute. Surfactants, disintegrants, and lubricants are usually added to the drug during the manufacturing process to increase the solubility and dispersibility of the active ingredient. In the case of enteric-coated tablets, hydrophobic lubricants and extreme pressure may be applied during the manufacturing process to slow physically the dissolution process. Dissolution is the rate of availability of the active ingredient for absorption or the time required for a given amount of drug to be released into solution from a solid dosage form. Dissolution time is usually measured *in vitro*, under conditions that simulate those

that occur *in vivo*, in experiments where the amount of drug in solution is determined as a function of time (Savjani et al., 2012; Rang et al., 2016). These processes permit the active ingredient to be released over a longer period of time and are critical to time-release drugs.

Once the drug has entered the GI tract and has been processed to either a liquid or a solid that has been thoroughly dissolved, the drug yet must pass through several lipid cell membrane barriers prior to reaching the circulatory or lymphatic system. Immediately adjacent to the lumen cell wall is an aqueous boundary layer or “unstirred water layer” which comprised of <5% mucus and 90% water (Carlstedt et al., 1985; Wood et al., 2018). This layer acts as a diffusion barrier to drugs, toxicants and chyme alike and represents another barrier to surmount in the absorption process. In addition to the unstirred water layer, there is another layer termed the “acid microclimate” which exists at the surface of the luminal cell membrane. The acid microclimate is maintained by sodium–proton exchangers in combination with several HCO_3^- -ion exchangers (e.g., the $\text{Cl}^-/\text{HCO}_3^-$ -exchanger) and other transporters (Artursson et al., 2007). Once the substance traverses the unstirred water layer and the acid microlayer, it reaches the surface of the membrane of the lumen and must face numerous variables such as physical forces, osmotic concentration, pH, digestive enzymes, other foodstuffs, endogenous biochemicals and commensal microbes hindering absorption (Bellmann et al., 2015).

There are two primary pathways for the substance to enter the cellular membrane: (1) through the cells (transcellular) or (2) between the cells (paracellular). The following section describes each of these absorption mechanisms individually.

TRANSCELLULAR TRANSPORT

Substances entering the cellular membrane via the transcellular route do so either passively or are actively transported. There are several types of transcellular transport processes including those that are completely passive, those that are active and those that do not fit into either category.

Passive Cellular Transport

Passive cellular transport expends no energy and is the most common route of absorption in the GI tract. It is dependent upon the permeability of the membrane and the surface area available for absorption. There are 3 types of passive cellular transport mechanisms that are found in the GI tract: (1) diffusion, (2) Osmosis, and (3) facilitated diffusion.

Diffusion

This type of transport shows low chemical specificity and does not involve a carrier or the expenditure of energy. The process involves the absorption of a substance across a concentration gradient from a membrane compartment of high concentration

to a compartment of low concentration. Diffusion only occurs when there is a concentration gradient of a substance separated by a membrane. The physics of diffusion follows Fick's first law of diffusion which describes the passive movement down a concentration gradient to equilibrate the substance in both compartments. Given enough time, the flow of substance will eventually result in homogeneity within the matrix, causing the net flow of substances to cease. Fick postulated that the flux of material across a given plane is proportional to the concentration gradient across the plane (Guenneau and Puvirajesinghe, 2013; Zhou et al., 2015). Mathematically this movement is described by the following equation:

$$F = -D \left(\frac{\partial C(x, t)}{\partial x} \right)$$

where F is the flux, D is the diffusion constant for the substance that is diffusing in the specific solvent, and $\partial C(x, t)/\partial x$ is the concentration gradient. The diffusion constant of a substance is also referred to as 'diffusion coefficient' and it is expressed in units of length²/time, such as $\mu\text{m}^2/\text{hour}$. The negative sign $[-]$ on the right side of the equation indicates that the substances flow in the direction of lower concentration.

This pathway is primarily for lipophilic substances. Fromm and Hierholzer (2000) have shown that different sections of the GI tract membrane have varying degrees of permeability because of thickness and fluidity of the basolateral membrane. Additional factors governing absorption with passive cellular transport include the available membrane surface area and transit time—both increasing proportionately with increasing value (Dreij et al., 2011; Feher, 2012).

Osmosis

Osmosis is the movement of water from a region of high water concentration through a semi-permeable membrane to a region of low water concentration—in a direction that tends to equalize the solute concentrations in the two regions. In cellular biology, this is usually when a solvent such as water flows into or out of a cell depending on the concentration of a solute such as salt. Thermodynamic theory indicates the concept of chemical potential and how the function of the water on the solution side is different from that of pure water due to the higher pressure and the presence of the solute counteracting such that the chemical potential remains unchanged. The virial theorem demonstrates that attraction between the molecules (water and solute) reduces the pressure, and thus the pressure exerted by water molecules on each other in solution is less than in pure water, allowing pure water to “force” the solution until the pressure reaches equilibrium (Borg, 2003). Water molecules—unlike other substances—tend to be drawn together by weak hydrogen bonds. This weak bonding makes the molecules stick to one another in an amorphous mass and their movement across is also referred to as bulk flow. Osmosis ultimately is the passive movement of groups of water molecules through cell membranes and requires no energy (Fisher et al., 2011).

Facilitated Diffusion

Facilitated diffusion or facilitated transport is a protein carrier-mediated process by which molecules or ions can pass through the cell wall via specific transmembrane integral proteins. If a particle is not lipid soluble and is too large to pass through the pores of the cell wall, then it uses the help of a carrier protein. An example is glucose, which is not only too large to enter the membrane but also insoluble in lipids. The process involves binding of the substance to a specific carrier protein. The complex binds to a receptor site within the cell which transports the entire complex through the cell membrane and releases it on the other side. Facilitated transport does not directly require chemical energy from ATP hydrolysis in the transport step itself; rather, molecules and ions move down their concentration gradient reflecting its passivity. It differs from simple diffusion in that it relies on molecular binding with a carrier protein, is saturable, and is significantly temperature dependent (Bauer and Metzler, 2013; Gomez and Klumpp, 2016).

Active Cellular Transport

There are many compounds whose movement across the mucosal epithelial membrane requires active assistance and the expenditure of energy. This is generally because the substance has a large molecular weight, is insoluble or is moving against a concentration gradient. Since these substances successfully traverse the membrane, a system of transport mechanisms has evolved to permit the movement of the numerous materials inside the mucosal epithelial cells. This process is characterized by selectivity and requires energy expenditure by the cell. The process of active transport moves molecules against their electrochemical gradient, a process that would not be possible if it were not coupled with an energy source—the hydrolysis of ATP. This coupling can be either primary or secondary. In the primary or direct active transport, transporters that move molecules against their electrical/chemical gradient, chemically hydrolyze ATP. In the secondary active transport or cotransport, transporters use energy derived from transport of another molecule in the direction of their gradient, to move other molecules in the direction which is against their concentration gradient. There is no direct coupling of ATP, but instead secondary transport relies upon the electrochemical potential difference created by pumping ions in/out of the cell (Urry et al., 2016). If there is an unequal distribution of charges across the membrane, then the difference in electric potential generates a force that drives ion diffusion until the charges are balanced on both sides of the membrane. In secondary active transport, the two molecules being transported may move either in the same direction (i.e., both into the cell) or in opposite directions (i.e., one into and one out of the cell). When the molecules move in the same direction, the protein that transports them is called a symporter; if they move in opposite directions, the protein is called an antiporter (Nelson and Cox, 2014).

Active transport processes have been identified for various ions, vitamins, sugars, and amino acids as well as some xenobiotic materials. The divalent-metal ion transporter (dmt) assists with the absorption of iron, copper, and zinc (Espinoza et al., 2012) while nucleotide transporters (nt) (Geisler et al., 2013; Sesma et al., 2013) and peptide

transporters (pept1, pept2) (Solcan et al., 2012) function in the transport of nucleotides and peptides, respectively. While these transporters seem to be quite different as they transport widely diverse substances, they are similar in many respects. There are characteristics which are exhibited by most active transport systems (Kapp, 2007, 2014):

1. The system is chemical-structural specific.
2. The system is carrier-mediated.
3. The transport system utilizes energy.
4. The system is competitive among other similarly structured chemicals that utilize the same transport system.
5. The system is transport-rate limiting either by saturation or competition and exhibits a transport maximum (T_m).
6. The system moves substances against concentration/energy gradients.

Direct Cellular Transport

Direct active transport or primary active transport is coupled directly to an exergonic reaction, which is primarily the hydrolysis of ATP. These transport proteins are termed “ATPases” or “ATPase pumps.” There are four types of transport ATPases:

1. **P-type ATPases**—The P type is a large family of membrane proteins that perform active ion transport across biological membranes. In these proteins, the energy-providing ATP hydrolysis is coupled to ion transport of one or two ion species across the respective membrane. This type includes H^+ ATPase of the GI tract epithelial cells (Rosenzweig and Argüello, 2012).
2. **V-type ATPase**—The V-type does not undergo phosphorylation. These types of ATPases are involved with vesicle pumps which pump protons into organelles of the EMS. V-ATPase is a highly conserved evolutionarily ancient enzyme with remarkably diverse functions in eukaryotic organisms (Cole, 2016; Kimball, 2018)
3. **F-type ATPase (also known as ATP Synthase)**—This F type is found only in bacteria, mitochondria, and chloroplasts. These ATPases are involved with proton transport in these organisms which use energy of ATP hydrolysis to drive ATP synthesis (Cole, 2016).
4. **ABC type ATPase**—The ABC type ATPases or “ATP Binding Cassettes” are transmembrane proteins which expose a ligand-binding domain at one surface and an ATP-binding domain at the other surface and are usually restricted to a single type of molecule. The bound ATP provides the energy to the ligand transfer. The human genome contains at least 48 ABC transporters including CFTR (Cystic fibrosis transmembrane conductance regulator and TAP (Transporter associated with antigen processing) (Wilkins, 2015; Kimball, 2018).

Indirect Cellular Transport

Indirect or secondary active transport which is characterized by the fact that the energy driving the system is that of two molecules—one going down its gradient drives the movement of the other substance against its gradient. But since the gradient is maintained by ATP hydrolysis, ATP is the indirect source of energy

for this process. Generally, the driving ion in this scenario is Na^+ with its gradient established by the Na^+/K^+ ATPase. There are two basic types of indirect active transport mechanisms: symport and antiport (Forrest et al., 2010; Kimball, 2018).

1. *Symport type transporter*

A symporter is an integral membrane protein that is involved in the transport of many differing types of molecules across the cell membrane. The symporter works in the plasma membrane and molecules are transported across the cell membrane at the same time, and is, therefore, a type of cotransporter. The transporter is called a symporter, because the molecules will travel in the same direction in relation to each other. In this type of indirect active transport, the driving ion (usually Na^+) is forced through the membrane pump in the same direction as a second molecule or ion. An example of this is the $\text{Na}^+/\text{glucose}$ transporter in which the transmembrane protein permits Na^+ ions and glucose to enter the cell together. The Na^+ ions flow down their concentration gradient while the glucose molecules are pumped against their concentration gradient. Eventually, the Na^+ is pumped back out of the cell by the Na^+/K^+ ATPase. This pump is used to actively transport glucose out of the GI tract and into the circulatory system (Youn et al., 2009).

2. *Antiport*

An antiporter (also called exchanger or cotransporter) is a cotransporter and integral membrane protein involved in secondary active transport of two or more different molecules or ions across a phospholipid membrane such as the plasma membrane in opposite directions. In this type of indirect active transport, the driving ion (also usually Na^+) diffuses through the pump in one direction and that transfer provides the energy for the active transport of another molecule in the opposite direction. An example of this type of pump is the H^+/K^+ ATPase which catalyzes the transport of H^+ out of the gastric parietal cell (toward the lumen of the stomach) in exchange for a K^+ ion which enters the cells (away from the lumen of the stomach) (Pak et al., 2013).

Endocytosis

Endocytosis is a form of active transport by which a substance gains entry into a cell by passing through the cell via invagination of its membrane to form a vacuole. The cell wall changes shape (invaginates) in such a way that it encloses the substance, then the wall re-fuses forming a vesicle that moves to the interior of the cell. This mechanism of absorption utilizes energy; therefore, it is considered a form of active transport. It is not believed that endocytosis is involved with the absorption of many drugs in a major way. Endocytosis is divided into three distinct types: (1) pinocytosis, (2) phagocytosis, and (3) receptor-mediated endocytosis (Miaczynska and Stenmark, 2008; Liu et al., 2009; Schmid et al., 2014; Porter, 2018).

Pinocytosis

Pinocytosis is Greek for “cell drinking” and involves the plasma membrane invaginating a volume of extracellular fluid and anything it contains including water, salts, biochemicals, and even soluble macromolecules. In this type of

endocytosis which is also termed “fluid endocytosis” and “bulk-phase pinocytosis,” small particles suspended in extracellular fluid are brought into the cell through an invagination of the cell membrane, resulting in a suspension of the particles within a vesicle inside the cell. These pinocytotic vesicles fuse with lysosomes to hydrolyze the particles. The invagination and subsequent restructuring of the cell wall require energy (Stillwell, 2016). Pinocytosis is used primarily for clearing extracellular fluids and as part of immune surveillance (Kruth, 2011; Abbas et al., 2016).

Phagocytosis

Phagocytosis is Greek for “to devour” and involves the plasma membrane invaginating a solid particle into an internal compartment known as a phagosome. In this type of endocytosis, the cell changes shape by sending out projections around the substance. These projections are called pseudopodia or “false feet.” The cell wall is attracted to the substance by a chemical (chemotaxis). The cell sends out projections in the membrane that contact the substance making a non-specific receptor ligand interaction. Subsequently, the membrane projections engulf the substance eventually re-fusing the cell walls forming an intracellular vesicle. Phagocytosis in an organism’s immune system is a major mechanism used to remove pathogens and unnecessary cellular debris. Upon ingestion of a pathogenic microorganism by a macrophage, it becomes trapped in a phagosome which then fuses with a lysosome to form a phagolysosome. Within the phagolysosome, enzymes and toxic peroxides digest the pathogen. Debris such as bacteria, dead tissue cells, and small mineral particles are all examples of objects that may be phagocytized. The resulting waste material is discharged from the phagocyte by exocytosis. This type of endocytosis is limited to certain specialized cells such as neutrophils, macrophages, and amoeba so phagocytosis is not involved in the absorption of drugs per se (Freeman and Grinstein, 2014; Bourne, 2017; Rosales and Uribe-Querol, 2017).

Receptor-Mediated Endocytosis (RME)

Receptor-mediated endocytosis (also termed “clathrin-mediated endocytosis”) is a process whereby cells absorb metabolites, hormones, other proteins—and in some cases viruses—by the inward budding of plasma membrane vesicles containing proteins with receptor sites specific to the molecules being absorbed (Lu et al., 2016). Specific receptor sites on the outer cell wall bind tightly to specific ligands on the outside of the cell. As that occurs, the cell sends out pseudopodia projections and engulfs the substance as described for phagocytosis. The difference is that the specific substances are very important to the cells survival, so the rate of absorption may be as much as 1000 times more efficient than pinocytosis. An example of this type of endocytosis is characterized by the uptake of iron. Iron is necessary for the cell’s survival. Iron is transported in the blood complexed to a protein called transferrin. Interestingly, cells have receptors for transferrin on their cell surface which specifically bind to transferrin. The iron complex is subsequently absorbed into the cell via endocytosis. Eventually, the iron is released for the cell’s use.

Since the receptor is specific, the cell can get the need amount of iron/transferrin complex even if the concentration is only a fraction of the content of the extracellular fluid. Cells can obtain cholesterol by the same receptor-mediated endocytosis (Sorkin and Puthenveedu, 2013; Kimball, 2018).

Exocytosis

Exocytosis is the active transport in which a cell transports molecules out of the cell by expelling them through an energy-dependent process. Exocytosis is the reverse of endocytosis but is a mechanism whereby the cell can release various substances such as secretions and toxins outside of the cell. Generally, in this case, membrane-bound vesicles (exocytic) move to the cell surface where they fuse with the cell wall. As they fuse with the cell wall, these vesicles turn inside out and empty their contents into the extracellular fluid. It is believed that the exocytic vesicles are created from endosomes that do not fuse with lysosomes or the endoplasmic reticulum or Golgi apparatus which take various cell products to the surface. This secretion is possible because the vesicle transiently fuses with the outer cell membrane. These types of events occur in the pancreas in the process of the secretion of pancreatic enzymes. Exocytosis is also a mechanism by which cells can insert membrane proteins (such as ion channels and cell surface receptors), lipids, and other components into the cell membrane. Vesicles containing these membrane components fully fuse with and become part of the outer cell membrane. In addition, certain cells lining the intestinal lumen synthesize fat which is discharged by exocytosis into lacteals (Ivannikov et al., 2013; Liang et al., 2017; Kimball, 2018).

PARACELLULAR TRANSPORT

The paracellular transport pathway refers to the transfer of substances across an epithelium by passing through the intercellular space between the cells (Karasov, 2017). While transcellular transport often involves energy expenditure, paracellular transport is unmediated and passive down a concentration gradient through the intercellular spaces between epithelial cells. The controlling gateway of the paracellular pathway is the tight junction, which is an apically located cell–cell interaction of lumen epithelial cells. The tight junction permits the passage of ions while restricting the movement of large molecules. The porousness of the tight junction complex is dependent upon several factors including the molecular size of the substance, the net charge of the compound, intra and extracellular calcium ion concentration, osmolarity, protein kinase inhibitors and a complex network of proteins including claudin, occludin, and the junctional adhesion molecule (JAM). Claudins are a group of about 20 tissue-specific tight junction proteins that are believed to be part of the structure that contains two extracellular loops and varying amino-acid residues and short intracellular tails (Fasano and Shea-Donohue, 2005; Murugan et al., 2015). Occludin is polypeptide that contains four transmembrane domains with two extracellular loops with internal amino and carboxy termini (Fasano and Shea-Donohue,

2005; Liu et al., 2009). Junctional adhesion molecule is an immunoglobulin that has been identified as a component of the tight junction necessary to allow the transmigration of monocytes through the paracellular space (Woodfin et al., 2011). Tight junctions possess a complex regulatory system which changes the network of tight junction proteins to the physiological needs of the organism. Tight junctions are estimated to be less than 0.1% of the total surface area of the intestinal tract. Further, the substances absorbed by this method are small hydrophilic molecules. It is believed that substances transported via the paracellular route are generally incompletely absorbed into the circulatory system. Hence the tight junctions are dynamic interactive structures that are critical in the physiology of absorption.

DISTRIBUTION

Distribution is the process by which an absorbed substance is reversibly transported through the circulatory or lymphatic system to various tissues throughout the body where it can react with various body tissues and/or endpoint receptors. Once a substance is absorbed into the circulatory system it is almost immediately circulated throughout the entire body. While the blood and lymphatic systems transport the substances quickly, the substances are not absorbed evenly throughout the body. The distribution of a drug among various tissues is dependent on vascular permeability, regional blood flow, cardiac output and perfusion rate of the tissue and the ability of the drug to bind tissue and plasma proteins, pH partitioning and its lipid solubility. A substance is quickly distributed in highly perfused organs such as the liver, heart, and kidney. It is distributed in smaller quantities through less perfused tissues such as muscle, fat and peripheral organs. The substance can be moved from the plasma to the tissue until the equilibrium is established (for unbound substance present in plasma) (Le, 2017; Porter, 2018).

Apparent Volume of Distribution

The apparent volume of distribution (also termed “volume of distribution,” or V_d) is the theoretical fluid volume required to contain the total amount of an administered drug at the same concentration that it is observed in the blood plasma. It is an important pharmacokinetic parameter that relates drug plasma concentrations to the amount of drug in the body and is important for drug loading dose and maintenance dose calculations. This measure is a reference for the plasma concentration anticipated for a given amount of substance required to produce that concentration in the plasma and is defined as the distribution of a medication between plasma and the rest of the body after oral or parenteral dosing (Wesolowski et al., 2016).

The mathematical expression of that relationship is described in the following equation:

$$V_d = \frac{Q}{C_p}$$

where Q is the total amount of substance in the body at the same concentration as that present in the plasma which is represented by C_p . The V_d is not a physiologic value and does not provide any information about the pattern of distribution since each substance is distributed within the body in a different way (Davis et al., 2011; Bourne, 2017).

Patterns of Distribution

Generally, drug distribution follows one of four basic patterns:

1. Substance remains bound within the vascular system such as those materials that tightly bind to plasma proteins.
2. Substance becomes uniformly distributed through the body water.
3. Substance is concentrated in one or more tissues that may or may not be the site of action.
4. Substance is non-uniformly distributed throughout the body based upon membrane permeability and lipid solubility with the greatest concentrations often found in the kidney, liver, and colon which is a reflection of the substance being eliminated from the body.

Water-soluble substances usually remain in the blood and interstitial space. Acidic substances tend to bind to various blood protein components such as albumin. On the other hand, lipid-soluble substances tend to collect in adipose tissue and rather than rendering it inactive tend to extend the effect of the substance due to the storage depot effect of these substances. Some other substances are tightly bound to liver and kidney tissues. Equilibrium between blood and the target tissue is reached more rapidly in highly vascularized areas than in poorly perfused areas. At equilibrium, substance concentrations in tissues and in extracellular fluids are reflected by the plasma concentration (Doogue and Polasek, 2013; Smith et al., 2015; Bourne, 2017; Kimball, 2018). The equilibrium pattern of distribution between the various compartments (described below) will depend upon:

1. The permeability across tissue barriers
2. Binding within compartments
3. pH partition
4. Fat:water partition.

Body Compartments

Body water is distributed primarily into the following major body compartments while the total body water as a percentage of body weight varies from 50% to 70% in women and men, respectively. Extracellular fluid comprises the blood plasma and contains approximately 4.5% of the body weight, while interstitial fluid is ~16% while lymph ~1.2%. Interstitial fluid (30%–40%) is the sum of the fluid contents of all cells in the body. Transcellular fluid (~2.5) includes cerebrospinal, intraocular, peritoneal, pleural, and synovial fluids as well as digestive secretions. Fat is ~20% (Rang et al., 2016). Within each of the aqueous compartments, substances usually

exist both in free solution and in bound form. Substances are transported via the circulatory system partially unbound and partly bound as noted previously. Proteins such as albumin chemically “lock” a substance which renders it pharmacologically inactive while the unbound portion of the substance is considered to be the free fraction and is the pharmacologically active portion of the substance. Plasma protein binding significantly influences both the distribution and the relationship between the pharmacological activity and the substance concentration in the plasma (Waterhouse and Farmery, 2012). As the number of available binding sites approaches saturation at higher substance concentration levels, other substances may be displaced. This displacement activity is a critical function in chemical interactions.

Rate of Distribution

The rate of distribution is primarily a function of membrane permeability and blood perfusion. Perfusion is the passage of fluid through the circulatory system or lymphatic system to an organ or a tissue. Membrane permeability is governed by the solubility of the substance (lipids can pass through the membrane quickly whereas water-soluble compounds distribute less quickly). Substances that are ionizable in the blood will increase the rate of transfer across the membrane (Cuenod and Balvay, 2013).

The blood perfusion rate is the rate at which the blood is distributed throughout an organ. Perfusion is measured as the rate at which blood is delivered to tissue, or volume of blood per unit time (blood flow) per unit tissue mass. The SI unit is m³/s/kg, although for human organs perfusion is typically reported in mL/min/g. The perfusion rate varies widely from one organ to the next as indicated in Table 5.6.

The mechanical and electrical properties of the heart together with the circulatory system determine the cardiac output, which ensures the transport of oxygen and

Table 5.6 Perfusion Rates Highest to Lowest

Organ	Perfusion Rate (mL/min/mL of tissue)	Percent of Cardiac Output
Kidney	4.0	22
Thyroid	2.4	1
Adrenals	1.2	0.2
Liver	0.8	27
Heart	0.6	4
Brain	0.5	14
Fat	0.03	4
Muscle	0.025	15
Skin	0.024	6
Bone	0.02	5

Source: Kapp, R.W., Gastrointestinal tract as major route of pharmaceutical administration—Chapter 5, in Gad, S.C. (Ed.), *Toxicology of the Gastrointestinal Tract 1st ed., Target Organ Toxicology Series*, CRC Press, Taylor & Francis Group, Boca Raton, FL, pp. 107–134, 2007; Shargel, L. and Yu, A.B.C., *Applied Biopharmaceutics & Pharmacokinetics*, 7th ed., McGraw-Hill Education, New York, 2016.

various nutrients to the tissues and the simultaneous removal of the waste products of cellular metabolism as well as the delivery of pharmaceutical substances. The regional distribution of cardiac output may vary markedly according to regional organ perfusion. The higher the flow of blood through the organ, the higher the concentration of a substance one can expect in that organ (Calzia et al., 2005).

Extent of Distribution

The extent of distribution is affected by several factors including lipid solubility, pH-pKa, plasma protein binding, and intracellular binding. Plasma protein binding is involved since the drug usually remains in the circulatory system rather than transferring out into a tissue. Extensive plasma protein binding will cause more drug to stay in the central blood compartment. Thus, drugs which bind strongly to plasma protein tend to have lower volumes of distribution (Smith et al., 2012). Binding sites can be for acidic substances (albumins) or basic substances (α 1, α 2, β 1, β 2 and δ globulins), any of which can determine how tightly bound the substance may be to a plasma protein. Although drugs are bound to many macromolecules, binding to plasma protein is the most common. Of these plasma proteins, albumin, which comprises 50% of the total proteins, binds the widest range of drugs. The degree of interaction between the substance and the protein molecules in the circulatory system is determined partially by electrostatic interactions, van der Waal's forces, and hydrogen bonding. Very small changes in binding can release enough unbound substance into the plasma to make significant changes in the effect at the receptor site. Mathematically the binding of a protein substance to a binding site is described by the following equation (Bourne, 2017):

$$D = [nP - rP] \xrightleftharpoons[\text{K}_d]{\text{K}_a} [\text{DP}]$$

where D is the concentration of free substance, nP is the total concentration of protein binding sites, and rP is the concentration of bound substance or bound protein with "r" substance molecules bound per protein molecule. Generally, there can be one to four binding sites per each protein molecule. However, there are many variations.

Intracellular Binding

Substances may also bind to intracellular molecules which can be the pharmacological endpoint receptors for certain substances. The overall binding rate is influenced by tissue proteins, the affinity to lipids, or the binding to DNA.

METABOLISM

The term "metabolism" is derived from the Greek word *metabolē* meaning "to change." These changes are the chemical changes which occur to a substance following entry into the body. These chemical biotransformations sustain life within the cells.

Enzymes located within specific tissues are responsible for changes in a substance's structure and subsequently altering the pharmacologic properties of the substance. Biotransformation of a xenobiotic usually occurs within a matter of minutes or hours. The primary location for metabolic changes is the liver. Other locations include the lungs, kidneys, gastrointestinal epithelium, and skin. Some substances can be changed into less toxic substances or can be prevented from metabolizing into toxic derivatives altogether hence the process can be an important defense mechanism. Unfortunately, the reverse is also possible: a substance can enter the body as a nontoxic entity and can be metabolized into a toxic derivative (Kapp, 2014; Lehman-McKeeman, 2015).

Phase I and Phase II Reactions

These metabolic reactions can be divided into two general types of reactions—Phase I and Phase II. In Phase I reactions, the structure of the toxicant is changed in a way that it is readied for additional changes during the next step in the process (Parkinson et al., 2015). Phase I reactions involve the formation of new or modified functional groups or cleavage and are nonsynthetic reactions which can neutralize substances in three ways:

1. Make the structure hydrophilic
2. Divide the substance into two or more less toxic substances
3. Transform the substance into an activated form that can be further metabolized by other enzymes

The majority of Phase I reactions are mediated by the cytochrome P-450 enzymes which oxidizes lipophilic toxicants and adds polar groups to the molecular structure. Phase I reactions tend to make the substrates more readily excreted from the organism by exposing the functional group and ready it to be changed further during Phase II. Some of these reactions result in bioactivation of the substance. There are four major biochemical reactions that occur in Phase I including:

1. Oxidation
2. Reduction
3. Hydrolyses
4. Mixed-function oxidase system

Next are the Phase II reactions which involve conjugation with an endogenous compound and are considered to be synthetic reactions (Parkinson et al., 2015). There are seven major biochemical reactions that occur in Phase II including:

1. Glucuronidation
2. Sulphation
3. Glutathione conjugation
4. Epoxide hydrolase
5. Acetylation
6. Methylation
7. Amino acid conjugation

Each of these reactions metabolizes specific types of activated substances by adding a molecule to the structure which forms an inactive or significantly less active water-soluble metabolite. The final product can be eliminated from the body either in the urine or the bile.

First-Pass Effects

The metabolic activity of the small intestine is referred to as “intestinal first-pass effect.” It is a phenomenon of drug metabolism where the concentration of a drug is greatly reduced before it reaches the systemic circulation. While the small intestine is primarily an absorptive organ, it also has the ability to metabolize drugs by many pathways involving preconjugation (Phase I) and conjugation (Phase II) reactions (van de Kerkhoh, 2007; Kleinschmidt and Delaney, 2011). Many of the enzymes present in the liver are also found in the small intestine; however, the enzyme concentration levels are considerably lower in the small intestine than in the liver (Lin et al., 1999). Certainly, a critical factor that plays a critical role in the amount of substance that is absorbed is a phenomenon termed “hepatic first-pass effect.” In this case, orally ingested substances are absorbed by the intestinal mucosal cells, enter the capillaries and veins of the GI tract as described in the previous sections, and are transported through the portal vein directly to the liver before entering the general circulation of the body. The absorbed substance is exposed to the liver before it is allowed to pass through to the body. If the substance is lipophilic, non-polar, and has a low molecular weight, it is absorbed through the GI tract mucosal cells (Sultatos, 2007). These types of substances are difficult to eliminate and can accumulate throughout the body to toxic levels. Many of these lipophilic substances are biotransformed into hydrophilic metabolites which do not easily enter the membranes of the potential target tissues. Endogenous materials such as bilirubin are also biotransformed into hydrophilic derivatives which are excreted into the bile and are ultimately eliminated in the feces.

Substances can also be biotransformed into derivatives that are more toxic than the parent compound. This activity is known as bioactivation and can be very toxic to the organism. This process is also called toxication and it has several pathways depending upon the chemical as follows:

1. Nucleophile formation
2. Electrophile formation
3. Free radical formation

In each of these scenarios, an electron-rich or electron-poor derivative is created that is very reactive and can cause significant toxicity to the organism (Gregus, 2015).

ELIMINATION

The elimination of chemical substances from the body involves any one of a number of processes by which a drug is eliminated (excreted) from an organism either in an unaltered form or chemically modified as a metabolite. The process results in eliminating

waste products of metabolism and other materials that serve no function to the organism. Hence, elimination is the sum of the processes of substance loss for the body. It is an essential process in all forms of life. The kidney is the primary excretory organ; other excretory organs include the liver, the skin, the lungs, or glandular structures, such as the salivary glands and the lacrimal glands. Elimination pathways include urine, tears, perspiration, saliva, respiration, feces, and bile (Kok-Yong and Lawrence, 2015).

Generally, chemical substances are eliminated from the body by two means: they are either metabolized to an alternative form or they are excreted via urine (Bardal et al., 2011). Both forms of elimination, however, often follow a first-order process with the rate of excretion and metabolism dependent on the amount of unchanged drug in the body. Most substances are metabolized prior to being excreted by one or more of the reactions described in the metabolism section above. Excretion is the removal of waste substances from the body fluids. The main organs of excretion are the kidneys and accessory urinary organs, through which urine is eliminated and the large intestine from which solid wastes are expelled. The skin and lungs also have excretory functions of eliminating water and salt (skin) and water and carbon dioxide (lungs). Other routes of excretion include bile, saliva, and milk. Substances are eliminated by several processes. Polar substances are filtered from the blood in the kidneys and removed into urine. These substances are excreted from the kidney by glomerular filtration and by active tubular secretion following the same steps and mechanisms as the products of intermediate metabolism. Substances that are filtered by the glomerulus are also subject to the process of passive tubular reabsorption. Glomerular filtration will only remove those substances or metabolites that are unbound to proteins present in blood plasma (free fraction) and many other types of substances (such as the organic acids) are actively secreted. Weak acids are excreted when the tubular fluid is too alkaline reducing passive reabsorption. The opposite occurs with weak bases. Non-polar substances are generally reabsorbed from the kidney or distributed into adipose tissue. The liver metabolizes many substances that are non-polar, producing compounds which are more polar and more easily excreted. Enzymes in the liver form conjugates with substances or hydrolyzes and oxidizes them. The kinetics of a substance can be markedly altered by changes in metabolism or excretion. These processes considered together result in the elimination of a drug (Doogue and Polasek, 2013).

Renal Excretion

Renal clearance is the total amount of a substance excreted over a specific time period. It depends upon all sources of renal excretion. There are three processes involved in renal clearance:

1. Tubular secretion (Suchy-Dicey et al., 2016)
2. Glomerular filtration (Jin et al., 2008)
3. Tubule reabsorption (Haraldsson, 2010)

Substances and/or their biotransformed products are transported to the kidney tubules. Most substances enter the kidney tubule by tubule secretion. Tubular

secretion is an active transport system consisting of two primary carriers—acidic for acidic substances and basic for basic substances. The tubules also permit small molecules and unbound chemicals to passively filter from the blood to the Bowman's capsule of the tubule. Large molecules and substances bound to plasma proteins such as albumin are not filtered and are not well excreted by this process which is termed “glomerular filtration.” Lastly, some substances that enter the kidney tubule may be reabsorbed back into the blood stream. This is termed “resorption” and it is a passive diffusion process requiring no energy. Since water is resorbed back into the blood from the tubules to conserve body fluids, the process also transports some of these molecules—hence they are resorbed along with the water (Felmlee et al., 2013; Bourne, 2017).

Another factor that influences the excretion rate includes urine pH. Generally, it is observed that drugs are weak acids and/or bases. Acidic substances are ionized more effectively in alkaline urine, whereas alkaline substances are more effectively ionized in acidic urine. Ionized substances are more soluble in water which permits efficient dissolution in body fluids and ultimately better excretion.

The plasma half-life or half-life of elimination is the time required to eliminate 50% of the absorbed dose of a substance from an organism. The difference in a substance's concentration in arterial blood (before it has circulated around the body) and venous blood (after it has passed through the body's organs) represents the amount of the drug that the body has eliminated or cleared. Clearance is generally expressed as the plasma volume totally free of the substance per unit of time, and it is measured in units of volume per units of time. Clearance can be determined on an overall, organism level (systemic clearance) or at an organ level (hepatic clearance, renal clearance etc.). The equation that describes this concept is as follows:

$$CL_0 = Q \times \frac{C_a - C_v}{C_a}$$

where CL_0 represents the organ clearance rate, C_a is the substance plasma concentration in arteriole blood, C_v is the substance plasma concentration in venous blood, and Q is the blood flow.

Renal clearance rate is determined by the degree of plasma protein binding since the substance will only be filtered out if it is in the unbound form; the degree of saturation of the transporters (active secretion depends on transporter proteins that can become saturated) or the number of functioning nephrons (Felmlee et al., 2013; Rang et al., 2016).

Biliary Excretion

Bile acids are synthesized from cholesterol in the liver and secreted into the small intestine where they facilitate the absorption of lipids and some fat-soluble vitamins. The vast majority of bile acids are reabsorbed from the intestine, returned to the liver via the portal venous circulation, and re-secreted into bile in a cycle termed the “enterohepatic cycle.” Over 90% of the intestinal bile acids are reabsorbed so less than

10% are excreted in the feces. Substances and their metabolites that are excreted in bile are actively transported across the biliary membrane. In the intestine, bile is reclaimed through a combination of passive absorption in the jejunum, by active transport in the distal ileum, and by passive absorption in the colon. Bile acids are actively transported in the terminal ileum by the well-characterized ileal apical sodium bile acid cotransporter (ASBT—apical sodium-dependent bile acid transporter). This sodium transporter moves bile acids from the lumen of the small intestine across the membrane and into the portal circulation (Sharifi and Ghafourian, 2014; Stringer, 2015).

Chemicals with molecular weights greater than 300 and those with both polar and lipophilic groups tend to be excreted in the bile. Conjugation with glucuronic acid is also generally excreted in the bile. In addition, substances secreted into the intestine undergo enterohepatic cycling upon hydrolyzation. Biliary excretion only eliminates substances that are not recycled in the enterohepatic cycle (Hosey et al., 2014; Stringer, 2015).

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CHAPTER 6

Gastrointestinal Function and Toxicology in Canines

Charles B. Spainhour

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INTRODUCTION AND OVERVIEW

According to Dorland's Medical Dictionary, gastrointestinal means "...pertaining to or communicating with the stomach and intestine..." The broadest and most encompassing definition of the gastrointestinal (GI) tract can include the mouth, throat, esophagus, stomach, duodenum, pancreas, jejunum, liver, ileum, cecum, colon, rectum, and anus. However, for the purposes of this chapter, the working definition of the GI tract will be the esophagus, stomach, small intestine and large intestine.

The major function of the GI tract is to process and absorb water and nutrients, while food is physically moved from the mouth to the colon, where nonabsorbable wastes are stored for periodic elimination. The mucosal surface of the GI tract is composed of a highly dynamic population of epithelial cells that are specialized for transmembrane absorption and secretion. This epithelial lining comprises a huge surface area approximating the size of a tennis court and interacts with food, water and xenobiotics from the external environment as well as with the intestinal microflora. The secretory and absorptive abilities of the mucosa facilitate digestion and nutrient uptake, all of which must be accomplished while keeping potentially harmful pathogens and mutagens at bay in the gut lumen. The epithelium allows the absorption of fluid, electrolytes and nutrients in health and the secretion of huge volumes of fluids and electrolytes in pathologic states. The lifetimes of epithelial cells in the GI tract varies with region and cell type, but can be almost as long as a week. Such a lifespan allows a period of environmental interaction with genes that may lead to the development of neoplasia. However, the rate of cellular turnover can occur as quickly as over a couple of days thereby facilitating rapid recovery of function following acute insult. It is important to note that the small intestine rarely develops epithelial neoplasia. However, the slower turnover of colonic epithelium, the slower movement of colonic luminal contents and the bacterial modification of chemical structures of xenobiotics in the luminal contents of the colon appear to support the existence of higher rates of neoplasia in the colon.

A very important and fundamental feature of the GI mucosa is the spatial segregation of the proliferative cellular compartment from the terminally differentiated cells, especially in the small intestine, where a gradient of differentiation exists from the depths of the crypts of Lieberkühn to the tips of the villi. This organization has a strong effect on the histology and pathophysiology of the gastrointestinal tract.

The gastrointestinal epithelial mucosa, while very dynamic in nature, also serves a barrier function. This activity is accomplished through both the physical integrity of the mucosal surface and the extensive population of resident immune cells. Clinical consequences of physical disruption of the mucosal epithelial layer can include blood loss, fluid loss, pathogenic invasion, impaired digestion, and altered nutrient absorption. The intestinal lymphoid system must maintain a balance between dampening immune reactivity at the mucosal surface in order to prevent the constant and unrestrained activation of the immune system and the amplification of an appropriate immune response when surface defenses have been breached. Derangements in this balance of suppression and stimulation can predispose the GI tract to inflammation.

Proper GI function also depends on the coordinated propulsion of food through the gastrointestinal space via smooth muscle contractions. Dysfunction of smooth muscle contraction can of itself result in major pathology of the gastrointestinal tract. The local and distant neural and endocrine factors that contribute to the regulation of intestinal motility are complex and the disruption of motility can alter the frequency of stool elimination and cause nausea, anorexia, abdominal pain, flatulence, constipation, and abdominal distension.

There is a tendency to view the GI tract as just a muscular tube with an epithelial lining but such a view would be very myopic and incorrect. The GI tract can be

affected directly or indirectly via secreted hormones, paracrine mediators, and its own enteric nervous system. The enteric nervous system contains 10–100 million neurons, an aggregation equal to the total number of neurons in the spinal cord. The GI tract's immune cells collectively make up the gastrointestinal tract's associated lymphoid system (GALT) and constitute the largest immune organ of the body.

Collectively, all of these systems must work together in a well-coordinated and harmonious fashion to permit the smooth integration of the functions of this complex organ system. However, this same complexity presents multiple foci of potential interruption of function and subsequent dysfunction. To fully appreciate and understand the potential for toxicity to and the dysfunction of the gastrointestinal tract, it is important to completely understand the structure, activities, and function of the GI tract.

GROSS AND MICROSCOPIC ANATOMY

The esophagus is the first part of the gastrointestinal tract [16,19,85,105,113,134,137,173,176,240,261,278,361,377]. It is a connecting tube between the pharynx and stomach. In the collapsed state and a dog of about medium size, it is approximately 30 cm in length and 2 cm in diameter. Due to its length, it is divided into cervical, thoracic, and abdominal portions. The esophagus is not uniform in either the thickness of its wall or the diameter of its lumen. The wall of the cervical portion averages about 4 mm in thickness, the thoracic portion about 2.5 mm thickness and the abdominal portion about 6 mm in thickness. As is suggested by the longitudinal folds (approximately 10) the esophagus is capable of a great degree of distension. The least expandable portions occur at the very beginning and end of the organ. The esophagus starts at the level of the axial vertebra and ends at the cardia of the stomach. A plicated ridge of mucosa, the *limen pharyngoesophageum*, which is most prominent ventrally, is the demarcation between the end of the pharynx and the start of the esophagus. The termination of the esophagus lies immediately ventral to the last thoracic vertebra. The cervical portion of the esophagus is ventral and to the left of the trachea. At its origin the esophagus starts to incline to the left, so that at the thoracic inlet, it usually is found to lie to the left lateral side of the trachea. However, individual variation is not uncommon and the cervical esophagus can be found to be left ventral or dorsal and left to the trachea. In proximity to the esophagus on either side are the common carotids, vagosympathetic nerve trunks, and internal jugular veins. The thoracic portion of the esophagus extends from the thoracic inlet to the esophageal hiatus of the diaphragm. At the point of the thoracic inlet, it usually is to the left of the trachea, but eventually obliquely crosses the left surface of the trachea to gain a dorsal position at about the same point as where the trachea bifurcates into bronchi ventral to the fifth and sixth thoracic vertebrae. The dorsal branches of the left and right vagal nerves run dorso-caudally across the sides of the esophagus and unite with each other on the dorsum of the esophagus at a point approximately two to four centimeters cranial to the dorsal part of the esophageal hiatus. The dorsal vagal trunk that is formed continues and passes through the dorsal part of the esophageal

hiatus. The ventral vagal trunk follows a similar course. The abdominal portion or terminal part of the esophagus is wedge-shaped. Dorsally, this portion of the esophagus immediately joins the stomach, but ventrally it notches the thin, dorsal edge of the caudate lobe of the liver.

The esophagus has four coats. Moving from the superficial to the deep, these are the fibrous, muscular, submucous and mucous coats. In the cervical region, the fibrous coat or adventitia blends with the deep cervical fascia. The adventitia of the thoracic and abdominal portions of the esophagus blends with the endothoracic and transversalis fascia, respectively. The esophagus is largely covered by pleura in the thorax and peritoneum in the abdomen. At those points where the esophagus is not covered by serosa, its adventitia blends with that of the organs with which it comes in contact.

The muscular coat is composed of two oblique layers of striated muscle fibers, an inner coat and an outer coat. While muscular fiber patterns are somewhat complex and possibly random in direction at the beginning and end of the esophagus, the main musculature of the esophagus is in the form of well-defined spiral fibers. These spiral fibers start at a point about 5 cm from the origination of the esophagus and end at a point about 5–10 cm from the cardia of the stomach. Fibers that are superficial on one side become ones that are deep on the other, kind of like a weaving pattern. These apparently continuous obliquely-oriented bundles spiral around the axis of the esophagus in such a fashion as to cross each other at right angles, eventually making up the two muscular coats for the organ. Lines of decussation are located both dorsal and ventral to the esophagus. In addition to the two oblique coats, several poorly developed groups of longitudinal fibers exist. The best developed of these bands are about 1–2 mm in width, located to the left and to the right of the esophagus and typically fade away to nothing on the caudal portion of the cervical portion of the esophagus.

The submucous coat loosely connects the mucous and muscular coats and this allows the relatively inelastic mucous coat to be cast into prominent longitudinal folds when the esophagus is in the contracted or nondistended state. Blood vessels, nerves, and mucous glands reside in this layer.

The mucous coat is composed of a superficially cornified, stratified squamous epithelium, which contains the openings at about one millimeter intervals of the esophageal glands. In the collapsed esophagus, this coat is notable for forming the large and numerous longitudinal rugae and folds that one observes upon gross examination. Cardiac glands are noted to occur in the distal part of the esophagus.

Most nerve branches to the esophagus are too small to be seen with gross examination and a degree of overlap or redundancy is known to exist. However, for each nerve source, a focal or primary area of innervation does appear to exist. The craniocaudal innervation of the esophagus involves as many as 23 paired spinal ganglia located between C1 and L2. But there appear to be two peak areas of innervation for the cervical sector of the esophagus, C2 to C6 and T2 through T4. Similarly, there are two peak areas of innervation for the thoracic region of the esophagus, T2 through T4 and T8 through T12. Generally speaking, the cervical portion of the esophagus is thought to be supplied by the paired pharyngoesophageal and paired

pararecurrent laryngeal nerves, the cranial thoracic region by the left pararecurrent laryngeal nerve and the caudal thoracic and abdominal portions by the vagal trunks.

The blood supply to the cervical portion of the esophagus is from small branches originating from the cranial and caudal thyroid arteries. The cranial two-thirds of the thoracic portion of the esophagus is supplied by the bronchoesophageal artery. The blood supply of the remaining portion of the thoracic esophagus comes from branches emanating from the dorsal intercostal arteries. The terminal portion of the esophagus receives its blood from a branch of the left gastric artery. The veins which drain the esophagus are basically a satellite vasculature of the arteries which supply it. However, veins which drain the thoracic portion of the esophagus for the most part empty into the azygous vein.

The stomach is a highly variable in size, musculoglandular organ located between and connecting the esophagus and small intestine. In its dilated state, it is the largest organ of the entire gastrointestinal tract. When empty, the stomach does not make contact with the abdominal wall, but when moderately filled, it presses against the xiphoid and left hypochondriac areas of the abdominal wall caudal to the liver. In its completely filled state, the stomach lies in contact ventrally with the xiphoid and umbilical portions of the abdominal wall as well as the right and left lateral portions of the abdominal wall and reaches to a point just caudal to the umbilicus. An imaginary axis placed through the stomach traces out the letter "C" rotated 90 degrees in a counterclockwise direction. It is therefore simple to visualize that the stomach is positioned essentially in a transverse fashion, but more to the left than the right of a median plane. The stomach forms a deep impression into the caudal surface of the liver; however, when the stomach is completely empty, it can be located cranially to the thoracic outlet.

The stomach does not increase in size in a uniform fashion as it accumulates ingesta. In fact, when the stomach increases its size as a result of filling, the first part that expands is the fundus. As the fundus grows in size, it pushes caudally and dorsally, displacing the liver ventrally. The second part of the stomach to fill and expand is the body, which is the largest part of the stomach and is capable of the greatest degree of expansion. As the body fills, the stomach moves caudally and ventrally, makes extensive contact with the abdominal wall and can crowd or displace the intestinal mass and spleen. The pylorus varies least in its position and shape as the stomach fills with ingesta and is the last to expand. Within the pyloric part, the pyloric antrum expands more than the pyloric canal. The most likely reason for this limited expansion is that the pyloric portion of the stomach is designed to work essentially as a magazine or ejection mechanism, by which the partly digested stomach contents (chyme) are squirted into the duodenum.

The capacity of the stomach is somewhat variable, ranging from 0.5 to 8 L of volume. Typically, the capacity of the stomach is considered to be normalized to 100–250 mL of volume per kilogram of body weight. It is noteworthy that the normal stomach is capable of adjusting its size and capacity with only minimal changes in intragastric pressure. The average empty stomach of a 15 kg dog weighs approximately only 100 g. Most commonly, food remains in a dog's stomach for from 10 to 16 hours. The function of the stomach is to accept and store food and partly mix

the ingesta with enzymes, mucus and hydrochloric acid coming from gastric glands. The inlet to the stomach is referred to as the cardia and the outlet of the stomach as the pylorus. The stomach is divided into four areas, the cardiac portion, the fundus, the body and the pyloric portion. The stomach also has two curvatures, the greater and lesser, and two surfaces, a visceral and parietal.

The visceral surface of the stomach is the convex outer surface that faces chiefly dorsally, but also caudally and to the right. It lies in contact with the left lobe of the pancreas and is separated from the intestinal mass and left kidney by a fat-filled peritoneal sheet known as the greater omentum. The parietal surface of the stomach faces cranially, to the left and ventrally. The greater curvature of the stomach is convex in shape and extends from the cardia to the pylorus. In a moderately filled state, the greater curvature is approximately 30 cm in length. The lesser curvature also runs from the cardia to the pylorus, but by the shortest path connecting the two parts. The lesser curvature forms an uneven concavity containing an angular notch, into which the papillary process of the liver juts. The pyloric antrum and the pylorus lie to the right of the papillary process of the liver and the body of the stomach lies to the left of it.

The cardiac portion of the stomach is that portion of the stomach that actually unites with the esophagus. While the four coats of the stomach and esophagus blend together well, there are differences between the muscular and mucous layers of each organ. The fundus is the large blind out-pocket of the stomach located to the left and dorsal to the cardia. The large middle portion of the organ is referred to as the body of the stomach. It extends from the fundus on the left to the pyloric region on the right. The shortest path that ingesta can take in moving from the cardia to the pyloric region of the stomach is known as the gastric groove. This path essentially follows the lesser curvature of the stomach. The pyloric portion of the stomach represents about the distal one-third of the stomach, as measured along the lesser curvature. The pyloric portion is lightly sacculated as it joins the body of the stomach to the duodenum and eventually assumes an irregular funnel shape as one moves toward the cranially-oriented pylorus. The initial part of the pyloric portion has expandable thin walls and is referred to as the pyloric antrum. The distal one-third of the pyloric portion referred to as the pyloric canal is contracted and bent, due to the presence of a heavy, encircling double sphincter. The presence of this musculature also leads to the formation of the narrowest confine of the cavity of the stomach. The exit from the stomach to the duodenum is through the muscular sphincter, the pylorus.

The stomach has four different coats, a serous coat, a muscular coat, a submucous coat, and a mucous coat. The serosa of the stomach is very thin and elastic and closely adheres to the stomach musculature by a small amount of subserous tissue. This serous coat covers virtually the entire surface of the stomach. The muscular coat is composed of an outer longitudinal and an inner circular layer of smooth muscle fibers to which is added a layer of oblique fibers in the region over the body of the stomach. The outer longitudinal layer is essentially continuous with the longitudinal layers of both the esophagus and duodenum. The inner circular layer of the stomach is better-defined and more specialized than is the

longitudinal layer and at the area of the cardia the circular layer is thickened to form the relatively weak cardiac sphincter. The inner circular layer is very well-developed in the pyloric canal. The pylorus is also surrounded by a distinct circular muscle referred to as the pyloric sphincter. The oblique fibers are adjacent to the submucosa and spread out in a pattern much like that of a fan. The submucous coat consists of a strong but thin elastic layer of tissue, which more firmly attaches to the mucosa than to the muscularis and contains the finer branches of the gastric vessels and nerves. The mucous coat consists of a columnar-type of surface epithelium, a glandular lamina propria and a lamina muscularis mucosae, with the latter consisting of irregularly woven or stratified muscular fibers. In the empty or slightly distended organ, the mucosa and much of the underlying submucosa are observed to be thrown into folds, known as the *plicae gastricae*. These folds are for the most part longitudinal in their orientation and very twisted and contorted in form, except in the area adjacent to the lesser curvature. In the area near the lesser curvature, the folds are less crowded and are relatively straight in form. In an empty stomach, the folds may approach 1 cm in height and lie closely adjacent to one another. The normal color of the mucosa in the body and the fundus of a fresh, perfused, normal stomach is pink to red-gray. In the pyloric portion of the stomach, the color is the same, but lighter in hue. It should be mentioned that color can vary with the degree of freshness of the specimen and with the adequacy of blood supply.

With some magnification, the mucosa can be observed to contain approximately 40 very small, mountain-like raised areas in every square centimeter of tissue. These areas are referred to as *areae gastricae*. Each of these small gastric areas in turn are stippled on their surfaces and sides with numerous minute, pinprick-like openings, termed the *foveolae gastricae*. These structures are deepest in the pyloric region, where they average about 0.68 mm in length. These structures gradually decrease in size as one moves toward the cardia and eventually disappear, so that none are observed to exist in the cardiac gland region. There are approximately one million foveolae in the stomach of the dog and each foveolae contains about 16 gastric glands opening into it. Folds, called *plicae villosae*, may be observed between the gland openings.

The stomach has numerous glands, termed generally gastric glands. These glands have necks, bodies, are branched and tubular in form and extend all the way to the lamina muscularis mucosae. There are three types of glands in the dog, cardiac glands, pyloric glands, and gastric glands proper. It should be noted that the gross divisions of the stomach do not match exactly the populations of glands of the same or similar namesake. While cardiac glands are typically found in a narrow area encircling the cardia, they can also be found to occur along the lesser curvature. Pyloric glands inhabit the pyloric part of the stomach. Approximately two-thirds of the gastric mucosa of the fundus and body of the stomach is occupied by gastric glands proper or fundic glands. However, between the gastric glands proper and pyloric glands lies a zone of intermediate glands. This intermediate zone is approximately 2–3 cm in width as observed on an incised, opened, and laid-flat stomach. These glands differ from each other by the types of cells that they contain

and hence the secretions that they produce. There are no strict lines of anatomic or histological demarcation separating the different types of glands found primarily in one area from those found in an adjacent area. Actually, the intermediate gland zone mentioned earlier is probably just typical of the merging of two different glandular populations.

The primary function of the glands of the cardiac, intermediate and pyloric regions is to produce mucus, but the gastric glands proper produce hydrochloric acid and the enzyme pepsin. It should be noted that lymphoid tissue is scattered throughout the mucosa of the stomach, with some extension of it penetrating deeply through the lamina muscularis mucosae into the submucosa.

There are mainly four different types of glandular cells found in the stomach mucosa. These are parietal, chief, mucous, and enterochromaffin cells. Parietal cells are the source of the hydrochloric acid in the gastric juice. Chief or zymogenic cells contain granules which contain pepsinogen, the precursor to the main gastric enzyme, pepsin. Mucous cells are found in the necks of the gastric glands, occupying spaces between the parietal cells and produce mucus. Enterochromaffin cells are mostly found in the proper gastric glands, somewhat in the pyloric glands and can also be found in the small and large intestines and pancreas. These cells are known by many different names, some based upon their histological staining characteristics, but all secrete polypeptides and biogenic amines. Alternate names for these cells include gastrointestinal endocrine cells, argentaffin cells, argyrophilic cells, and gastroenterohepatic cells (GEP).

The nerve supply of the stomach comes from parasympathetic fibers running from the vagi and by sympathetic fibers coming from the celiac plexus. Sympathetic fibers arise from the celiacomesenteric plexus and reach the stomach by traveling on the numerous gastric branches of the celiac artery. Sensory innervation of the stomach comes from about 25 paired spinal ganglia (C2–L5), with peak innervation of the stomach coming from the area T2 through T10. The dorsal vagal trunk sends branches to the lesser curvature and ventral wall of the stomach. The ventral vagal trunk sends out small branches to the pylorus and the lesser curvature of the stomach.

There are two main set of arteries providing a blood supply to the stomach, the left and right gastric arteries. These vessels run along the lesser curvature and the right and left gastroepiploic arteries, which travel along the greater curvature. The veins draining the stomach are satellites of the arteries supplying the organ. Blood leaving the stomach enters the liver via the portal vein and the lymphatic vessels of the stomach drain into the hepatic lymph nodes.

The small intestine consists of three parts, the duodenum, the jejunum, and the ileum. The initial portion of the small intestine is a short, relatively fixed in place loop called the duodenum. The next segment is freely movable and is known as the jejunum. The very short terminal portion is termed the ileum. The intestine extends from the pylorus of the stomach to the ileocecal orifice, which marks the start of the large intestine. The small intestine is the longest part of the gastrointestinal tract, having an average length in a normal living dog of approximately three and a half times the length of the body. It should be noted that the length of the small intestine

in the dead dog is significantly longer, due to a cessation of peristaltic contractions and a loss of muscular tone.

The duodenum is approximately 25 cm in length and originates in the upper right hypochondriac region opposite the ninth intercostal space. Running in a caudal fashion to the tuber coxae, the duodenum then turns back upon itself and travels cranially and obliquely to the left, eventually connecting with the jejunum at a position to the left of the root of the mesentery just beneath the spine. The duodenum can be subdivided into four different portions, the cranial, the descending, the caudal and the ascending portions. Both the pancreatic and bile ducts open into the duodenum and accordingly in this area the acid contents of the stomach are then mixed with the alkaline secretions coming from the pancreas and the liver along with the small intestine itself. The duodenum originates from the pylorus in such a fashion that its right wall is longer than the left wall, which results in the formation of the cranial duodenal flexure. This portion of the duodenum lies in contact medially with the pancreas, ventrally with the stomach and dorsally and laterally with the liver. The descending portion of the duodenum moves caudad and is approximately 15 cm in length. The right lobe of the pancreas is dorsal to it and at its caudal extent the cecum is medial to it. The caudal portion of the duodenum connects the descending portion and the ascending portions is approximately 5 cm in length and typically located ventral to the sixth lumbar vertebra. Ventral to this segment is the terminal portion of the ileum and the jejunum. As the colon and urinary bladder fill, this portion of the duodenum can be pushed cranially. The curvature of the caudal portion is not sharp, but rather gradual in nature. The ascending portion of the duodenum is observed to course cranially, obliquely and to the left from this caudal flexure. Dorsal to this segment of the duodenum are the ureters, caudal vena cava, aorta and various nerve trunks, while ventral to it are coils of the jejunum. On the left, it lies in close proximity to the descending colon and eventually terminates in a ventral curve to form the duodenojejunal flexure.

By far, the largest amount of the small intestinal mass is made up of the jejunum and ileum. The jejunum starts with the duodenojejunal flexure, which is located just caudal and to the left of the root of the mesentery. The jejunum is ventral and caudal to the empty stomach. Dorsal to it are the pancreas, kidneys, large intestine, aorta, and vena cava. The ileum ends as the ileal papilla, opening into the initial portion of the ascending colon. This papilla has an orifice and sphincter muscle. The orifice is typically located between the descending and ascending portions of the duodenum. The jejunum and ileum are very mobile and probably the most mobile portions of the gastrointestinal tract. This is a result of the jejunum and ileum being suspended from the cranial portion of the sublumbar region via a large drape-like sheet of tissue called the mesentery. There are no distinguishable gross or microscopic features in the walls of the small intestine that definitely demarcate the division between the jejunum and ileum, but there are significant differences between the mucosa of the jejunum and the mucosa of the ileum. Typically, for the dog, the ileum comprises approximately the last 15 cm of the small intestine. By convention, only the terminal and highly thickened and contracted part of the small intestine is considered to be the ileum. One easy way to identify the ileum is to look for the ileocecal fold and its

antimesenteric (opposite the side of the attached mesentery) ileal vessels. The ileocecal fold is a narrow (1 cm in width) but long (2–30 cm) piece of peritoneum that connects the ileum to the cecum.

As with other parts of the gastrointestinal tract, the small intestine has four different layers or coats, serous, muscular, submucous, and mucous. The serous coat covers virtually the entire small intestine and is peritoneum. The muscular coat consists of a thin outer longitudinal coat and a thicker inner circular layer of smooth muscle. The submucous coat resembles that of the stomach and large intestine. It loosely interconnects the muscular and mucous layers and smaller nerves; lymphatics and blood vessels are located within it. The mucous coat has a velvet-like appearance throughout the entire small intestine, which results from the presence of a large number of intestinal villi. These villi significantly increase the absorptive surface area of the small intestine, as the ratio of mucosal area to serosal area for the entire small intestine for the canine is approximately 8.5 to 1. There are two different types of cells comprising this surface: one produces mucus and is called a goblet cell and the other type is a columnar-type cell, which functions in absorption. The deeper part of the mucosa contains intestinal glands and lymphoid tissue. Of these glands, duodenal glands in the dog are different from general intestinal glands in that they closely resemble the pyloric glands of the stomach and are located only around a narrow zone of the duodenum contiguous to the pylorus. While the glands reside mostly in the submucosa, portions of them may extend into the lamina propria of the mucosa. With regard to the lymphoid tissue, in about 22 different areas throughout the small intestine, the lymphoid tissue is aggregated together to form structures referred to as follicles. These follicles are readily visible when inspecting the intestine from the serosal surface in a distended bowel and are more frequently found in intestinal side walls as opposed to those portions of the wall attached to or opposite the mesentery. Many of these follicles are found in the duodenum and they are persistently found throughout the entire length of the small intestinal tract. These follicles are reasonably well-defined, circumscribed elevations that are approximately 2 cm by 1.5 cm in size.

Nerve fibers to the small intestine arise from the vagus and splanchnic nerves, via the celiac and cranial mesenteric plexuses. The small intestine receives its sensory innervation from as many as 15 paired ganglia (T2 through L3), with peak innervation coming from T6 through L1.

The duodenum receives its blood supply from both the cranial and caudal pancreaticoduodenal arteries. The jejunum receives its blood supply from approximately 12 to 15 jejunal arteries, which originate from the cranial mesenteric artery. The ileum receives its blood supply on its antimesenteric side via branches extending from the ileocecal artery and on the mesenteric side by the accessory cecal artery. It should be mentioned that the duodenum receives a richer blood supply than the ileum. Perhaps this is because the duodenum produces more fluid than the ileum, an amount approaching ten times as much. The veins draining the small intestine are satellites of the arteries supplying the organ. Lymph vessels from the duodenum drain into the hepatic lymph nodes and the variably present duodenal lymph node, while lymphatics from the jejunum drain into the right and

left mesenteric lymph nodes. The ileum's lymphatics empty into the right and left mesenteric lymph nodes and the colic lymph nodes.

The large intestine of the dog more closely resembles that of man than that of other domestic animals. It is of a slightly larger diameter than that of the small intestine, relatively short, has no striking structural features and is relatively unspecialized in nature. Its most important functions are the absorption of water and electrolytes from the fecal stream, conversion of the fecal stream into solid mass, fermentation, and storage of the fecal stream until it can be expelled. We speak of fermentation in the large intestine, but it does not occur to the degree found in ruminants. The large intestine harbors numerous bacteria, especially bacilli, and these microbes can digest substances like cellulose and perform various biochemical transformations. End products of the activities of various microbes residing in the large intestine include Vitamin K, Vitamin B₁₂, thiamin, and riboflavin. The production of Vitamin K is critical to the organism because the amount of this vitamin present in normal food is insufficient to maintain a normal blood coagulation profile. The large intestine can be subdivided into the cecum and colon.

The cecum is typically considered to be the first part of the large intestine, but it should be emphasized that in the dog, the ileum communicates with the colon and not the cecum. The cecum is just a blind sac extending off of the proximal portion of the colon. The cecum can be of either a sigmoid or corkscrew shape with a large U-shaped bend extending to the left from its ileal attachment. The cecum is situated to the right side of a median plane and is generally partially encircled by the duodenal loop. It is generally intimately applied to the coils of the jejunum and ventral to the second to the fourth lumbar vertebrae. The openings of the cecum and ileum are in close apposition to each other, being only approximately 1 cm apart. In a normal dog, the cecum is approximately 2 cm in diameter at its colic end, 5 cm in length and ends as a blunted cone of approximately 1 cm diameter. The middle portion of the cecum is the body of the cecum. The cecum is mobile and can assume a variety of different orientations within the abdomen, but typically is aimed transversely or caudally and ventrally. It is connected to the ileum via peritoneum and is capable of expanding to twice its normal size. It is only able to communicate with the colon through the cecocolic orifice, which limits access via the cecocolic sphincter, a specialized portion of the inner circular muscular coat.

The colon is generally divided into three different portions and their interconnecting flexures. The subdivisions are ascending, transverse and descending. This organ is approximately 2 cm in diameter and 25 cm in length. The colon occupies the dorsal part of the abdominal cavity and because of a shorter sheet of mesenteric attachment does not shift around as much as the small intestine. The colon is shaped much like an inverted letter J. The small arm of the letter J represents the ascending colon, the curved portion of the letter J the transverse colon and the long arm of the letter J the descending colon. The ascending colon is on the right side of the body, the descending colon on the left side and the transverse colon is cranial to the root of the mesentery. The ascending colon and transverse colon are connected via the right colic flexure and the descending colon and transverse colon connected via the left colic flexure. The ascending colon originates at the ileocolic sphincter and is typically about 5 cm in length.

From its origin, the ascending colon travels in a cranial fashion to its terminus at the right colic flexure. The ascending colon lies ventral to the right kidney and is bound on the right by the descending duodenum. The jejunal mass is generally ventral and to the left of the ascending colon. Cranially, the ascending colon touches the stomach. It should be noted that in the dog it is not uncommon for the ascending colon to be absent or found lying in a different spatial relationship to other organs. In cases of altered geography, the positional change has resulted from a failure of the entire gut to adequately rotate about the cranial mesenteric artery as should normally happen during development. The transverse colon is approximately 7 cm in length and travels from right to left in a slightly curved fashion, cranial to the cranial mesenteric artery and the root of the mesentery. The transverse colon is cranial and dorsal to the left limb of the pancreas and cranial and ventral to the stomach and is also not uncommonly found to be absent or lying in a spatial relationship to other organs that is different from normal. The descending colon is approximately 12 cm in length and traverses from the left colic flexure to and through the pelvic inlet, at which point it merges without gross distinction to the rectum. The descending colon travels in a relatively straight line along the left lateral abdominal wall, from approximately the left costal arch to the sacrum and also lies in close apposition to the left kidney. The ascending portion of the duodenum lies medial to the descending colon and the urinary bladder lies ventral to its most terminal portion.

The large intestine has the same four coats as the small intestine, mucous, sub-mucous, muscular and serous. The serous coat resembles that of the small intestine and while the muscular coat is similar to that found in the small intestine, is uniform in its thickness and consists of a thin outer longitudinal coat, a thicker inner circular layer of smooth muscle if observed to be present. The submucous coat does not differ significantly from that observed in the small intestine and small nerves, lymphatics, and blood vessels are located within it. The mucous coat, however, is significantly different from that observed in the small intestine as there are no intestinal villi and no aggregations of lymphoid tissue. However, solitary lymph nodules do exist and can be observed when viewing the dilated gut from the serosal aspect. Folds occur in the large intestine, but are only noticeable when it is highly contracted, as a minimal amount of distension is quite sufficient to remove them. These folds can be either circular or longitudinal in orientation, depending upon the type of contraction that the large intestine has undergone. The intestinal glands of the large intestine are longer and straighter than those found in the small intestine. There is also a significantly greater population of mucus-producing cells in the large intestine than found in the small intestine. A columnar epithelium lines the intestinal glands and is continuous with that which lines the mucosal surface of the lumen of the gut.

The nervous supply of the large intestine arises from vagal general visceral afferents and vagal parasympathetics, with sympathetic fibers also supplying the large intestine, via the cranial mesenteric plexus.

The large intestine receives its blood supply from the caudal mesenteric and colic arteries. The veins draining the large intestine are satellites of the arteries supplying the organ with lymphatics from the jejunum draining into the mesenteric and right, middle and left colic lymph nodes.

HISTOPHYSIOLOGY AND SECRETORY ACTIVITY

Numerous glands are located throughout the entire length of the gastrointestinal tract and the secretions from these glands include enzymes to accomplish digestion and mucus for protection and lubrication [2,6,8,72,86,93,94,103,111, 112,118,122,139,140,157,158,164,184,200,203,219,231,232,235,246,285,322–324, 332,338,342,344,389,398,402,403,409,415]. The nature of the secretions can also vary with the type of luminal contents that are present. Production is in response to the presence or anticipation to the presence of food in the alimentary canal and interestingly only in amounts sufficient to perform and complete their intended function.

The production of mucus is especially important. In its purest form, mucus is a thick secretion composed of various glycoproteins, water, and electrolytes. While the character of mucus can change depending upon which region from the gastrointestinal tract it is produced, certain characteristics persist that make it an ideal lubricant and protectant. Mucus has surface tension characteristics which promulgate a strong, even coating ability of and adhesion to not only intestinal luminal contents but also gastrointestinal epithelium. This material has a low resistance to slippage and permits the facile sliding of material along or past the gastrointestinal epithelium. It is resistant to digestion by gastrointestinal enzymes and due to the chemical properties of inherent glycoproteins is capable of providing buffering action against both acid (hydrochloric acid) and alkali (bicarbonate ion). Mucus contains bicarbonate ion which contributes to the neutralization of hydrochloric and other acid. Finally, the presence of mucus causes the fecal stream to adhere to itself, causing the consolidation of fecal particles into a fecal mass.

The glands that produce these different secretions are various and in the stomach and upper portion of the duodenum the glands that produce acid and pepsinogen are observed to be deep and tubular in shape. The intestinal epithelium has deep invaginations that reach down to the submucosa. These deepened pits are referred to as *crypts of Lieberkühn* and contain specialized secretory cells. Mucus or goblet cells are also widely prevalent and they respond to stimulation or irritation of the gastrointestinal epithelium with the production of mucus. The mucus that is produced by these glands extends all over the surface of the stomach and remaining gastrointestinal tract and protects the mucosal epithelium from injury due to frictional abrasion, the caustic effects of acid and the degradative effects of various enzymes.

As mentioned previously, the pure mechanical presence of food in the gastrointestinal tract is in and of itself a sufficient stimulus to initiate secretory activity of the various glands in the area that the food occupies as well as adjacent areas of the gastrointestinal tract. This stimulation is the result of direct contact of food with the gastrointestinal epithelium and the glands themselves. Distension of the gut wall, chemical interaction and the physical presence of material can also activate the enteric nervous system. The resulting reflexes stimulate both mucous cells on the epithelial surface of the gastrointestinal tract and glands located deep in the gastrointestinal tract wall to increase their levels of secretory activity. Stimulation of parasympathetic nerves innervating the gastrointestinal tract also produces an increase of secretory activity. This response is especially vigorous in the upper

portion of the gastrointestinal tract, where vagal parasympathetic nerves affect esophageal glands, gastric glands, the pancreas and Brunner's (mucus producing) glands of the duodenum. Similarly, pelvic parasympathetic nerves stimulate glands in the distal part of the large intestine. Secretion in the remainder of the small and large intestines occurs essentially in response to local neural and hormonal stimuli. Stimulation of sympathetic nerves in some parts of the gastrointestinal tract can result in a small degree of increase in secretion, but sympathetic stimulation also causes vasoconstriction of the blood vessels supplying the epithelium and glands. Because of this restriction of blood supply, sympathetic stimulation, in the presence of parasympathetic or hormonal-induced stimulation will generally result in a decrease of secretory activity.

Mention has been made to the importance of various hormones in the regulation of the amount and character of gastrointestinal secretions. These hormones are polypeptide or polypeptide-like and released from the mucosa of the gastrointestinal tract in response to the presence of food. Upon release, the hormones are absorbed into the blood, where they are carried to different glands and secretion is stimulated. The complete picture of hormonal stimulation of gastrointestinal secretion is quite complex and still not totally elucidated.

All of the steps of the processes for glandular secretion are not completely known, but there are some basic steps which undoubtedly apply in all cases. Secretory substances are produced in the endoplasmic reticulum and Golgi complex of a glandular cell using adenosine triphosphate generated by mitochondria and specific nutrients absorbed or actively transported into the cell. Ribosomes attached to the endoplasmic reticulum are essential for the formation of protein material. Secretory materials move through the endoplasmic reticulum to the Golgi apparatus, where they are packaged in special secretory vesicles. The vesicles are then moved to and stored in an area of the cell contiguous to a portion of the cell's membrane from which secretion will take place. Appropriate nervous or hormonal stimulation increases the permeability of the cell membrane to calcium, which enters the cell. The presence of this increased local concentration of calcium causes the membranes of the secretory vesicles to fuse together with the cell membrane, break open and release their contents into the extracellular area. However, for this secretion to be effectively delivered permitting complete function, the presence of sufficient water and electrolytes is necessary to wash or carry away the organic substance(s) secreted and released through the secretory border of the cell. Although not without contention, it is generally believed that this washing or sweeping activity is accomplished in response to appropriate neural stimulation. Signals from nerve endings terminating on the bases of glandular cells cause the active transport of chloride ions to the interior of the cell through the basal portion of the cell membrane. The build up of negatively charged ions inside the cell in turn causes positively charged sodium ions to also move into the cell in order to preserve electric neutrality. The accumulation of sodium and chloride ions within the cell drives the accumulation of water inside the cell via osmosis. The cell swells and the interior hydrostatic pressure increases, which eventually then causes small breaks in the cell membrane along the secretory

aspect, which permit the movement of water and electrolytes out of the cell. This rush of water and electrolytes moves the secreted organic material away from the border of the cell.

Although the secretion of saliva will not be specifically discussed here, as it is beyond the scope of this chapter its production is nonetheless a very important component for the normal function of the gastrointestinal tract. If saliva is not secreted, food becomes very difficult to swallow, even with the administration of inclusion of copious amounts of water. Saliva contains large amounts of potassium ions and bicarbonate ions, lesser amounts of sodium and chloride ions and typically a range of pH between 6.0 and 7.0. There are also two different types of protein secretions in saliva, serous, and mucous. The serous portion contains ptyalin, which is an alpha-amylase carbohydrate digestive enzyme, and the mucous part contains the surface protectant mucin.

The main esophageal secretion is mucus, the character of which changes with region of the esophagus. Mucus secreted by the upper portion of the esophagus acts essentially as only a lubricant, but mucus arising from the distal portion of the esophagus must also provide protection from refluxing acidic gastric juices in addition to providing lubrication. To accomplish this, the main body of the esophagus is populated with simple mucous glands, but at the gastric end of the esophagus the glands are of a different type and complex in nature.

With regard to the process of secretion in the stomach, it is important to realize that there are three phases of gastric secretion, a cephalic phase, a gastric phase and an intestinal phase. In the cephalic phase, the sight, odor, taste or memory of food can cause stimulation of the hypothalamus, amygdala or/and cerebral cortex, which in turn sends signals via the vagus nerves to the stomach so that gastric secretion initiates before food even enters the stomach. The relative contribution to gastric secretion of this phase is approximately one-fifth of the total amount of secretions produced with the ingestion of a bolus of food. The gastric phase is initiated as food enters the stomach with gastric juice being secreted in response to local enteric reflexes, vagal-vagal reflexes, and the gastrin hormonal cycle. This phase of gastric secretion can generate over a liter of gastric secretions within the course of a day and contribute the majority of total gastric secretions. The last or intestinal phase starts commences with the entry of food into the duodenum. As the duodenum undergoes distension, small amounts of gastrin are released by G or gastrin cells located in the duodenal mucosa. Gastrin causes the stomach to continue to secrete a small amount of gastric juice to further digestion.

The entire surface of the stomach is populated with mucous cells and collectively these cells produce a continuous, very viscous, relatively insoluble coating of alkaline mucus that is approximately 1 mm in thickness and acts as a lubricant and protectant preserving the gastric mucosa from abrasive or acid-induced injury. While it should be obvious, it is important to repeat that as a result of the presence of mucus, the gastric mucosa is never actually exposed to ingesta and the secretions of acid and enzymes produced by other glands in the gastric mucosa. Indeed, even the slightest direct contact of the mixture of food, acid, and enzymes with the gastric mucosa will trigger the secretion of additional amounts of viscous, alkaline mucus.

The stomach has two other common and different types of glands in addition to the mucous glands and these are the gastric and pyloric glands. Pyloric glands, located in the antral portion of the stomach, are tubular in shape and secrete mainly mucus for protection of the pyloric gastric epithelium along with the enzyme pepsinogen and the hormone gastrin. Gastric or oxyntic glands are also glands that are tubular in shape, located in the mucosa of the body and fundus of the stomach and produce pepsinogen, mucus, intrinsic factor, and hydrochloric acid. In terms of relative populations, gastric glands outnumber pyloric glands by a ratio of almost four to one. The typical gastric gland is composed of parietal, peptic and mucous neck cells. Mucous neck cells produce mostly mucus and small amounts of pepsinogen. Peptic or chief cells produce large quantities of pepsinogen. Parietal or oxyntic cells secrete hydrochloric acid and intrinsic factor. The parietal cells on their secretory borders have large numbers of deep, highly-branched invaginations referred to as canaliculi, which are freely open to and communicate with the lumen of the gastric gland. Hydrochloric acid formed in the recesses of these canalicular lumina moves through these spaces to mix with other secretions of a gastric gland and then eventually mix with gastric contents. The acid solution secreted by parietal cells contains approximately 150 mmol of hydrochloric acid per liter. This concentration is essentially isotonic with body fluids, but at solution pH of about 0.8 and this level of hydrogen ion concentration is higher than that found in arterial blood by a multiple of approximately three million. Although several proposed mechanisms exist with regard to the production of hydrochloric acid, one commonly accepted mechanism is presented here. Chloride ions are moved via an active transport process from the cytoplasm of a parietal cell into the lumen of the canaliculus. While this process proceeds, sodium ions are actively transported from the lumen of the canaliculus. This combined action creates an electronegative sink, which consequently attracts the diffusion of potassium ions and some sodium ions from the cytoplasm of the parietal cell into the lumen of the canaliculus. Thus the contents of the canaliculus early in a secretory cycle are essentially that of potassium chloride with a small amount of sodium chloride. Water normally exists in equilibrium with its component hydrogen and hydroxyl ions and a membrane-bound H^+ , K^+ -ATPase pump actively transports and exchanges hydrogen ions from the cytoplasm into and potassium ions out of the canalicular lumen while at the same time a separate sodium ion pump removes sodium ions from the canalicular space. The end result is that eventually all of the sodium and potassium ions that were initially in the canaliculus are replaced by hydrogen ions. Water persistently moves via osmosis into the canalicular space as a result of the secretion of ions into the canaliculus. The resulting concentrated hydrochloric acid solution is then secreted outward through the open end of the canaliculus into the lumen of the gastric gland. The final hydrochloric acid solution secreted into the gastric space contains approximately 150 milliequivalents per liter of hydrogen chloride, 15 milliequivalents per liter of potassium chloride and a small amount of sodium chloride. In the final step of this cycle, carbon dioxide, an end product of normal metabolism, with the assistance of carbonic anhydrase combines with hydroxyl ion in the cytoplasm to form bicarbonate ions. Bicarbonate ions diffuse out of the cytoplasm of the parietal cell into the extracellular fluid of the body in

exchange for the chloride ions that enter the parietal cell and are eventually secreted into the canaliculus. In this fashion electrical balance is maintained between the parietal cell and its extracellular microenvironment.

Various types of pepsinogen are secreted by the peptic or chief cells and mucous cells of the gastric glands, but despite differences, all pepsinogens perform the same biochemical activity. Nascent pepsinogen (molecular weight approximately 42,000) has no enzymatic activity, but when it comes in contact with hydrochloric acid, it becomes activated to pepsin (molecular weight 35,000). This process is accelerated in the presence of previously formed pepsin. Pepsin is a proteolytic enzyme exhibiting maximal hydrolytic activity within the pH range of 1.8–3.5 and essentially inactive at pH values above 5.0. Other enzymes are found in gastric juices and these include, but are not limited to gelatinase, gastric lipase and gastric amylase; however, these enzymes play relatively minor roles in the process of digestion.

The substance intrinsic factor, mentioned earlier is essential for the absorption of Vitamin B₁₂ in the ileum and is secreted by parietal cells along with hydrochloric acid. Pyloric glands are basically identical to the gastric glands, but instead contain mostly mucous cells and contain scant to no numbers of parietal and peptic cells. The pyloric glands do secrete the hormone gastrin and a small amount of pepsinogen along with a large amount of thin nonviscous mucus, which aids the movement of ingesta along the gastrointestinal tract.

Essential neurotransmitters and hormones that can bring about secretion by the gastric glands are gastrin, histamine, and acetylcholine. These substances bind to specific receptors, the process of which triggers the initiation of a cascade of events culminating in secretion. Gastrin and histamine stimulate the production of acid but have little other stimulatory effects on secretory cells of the stomach. Acetylcholine, on the other hand, stimulates secretion by all cell types in the gastric glands and secretory products can include hydrochloric acid, pepsinogen, and mucus. The secretion of acid from parietal cells is under the control of both neural signals and the presence of hormones. Parietal cells operate in close association with histamine-producing enterochromaffin cells, which are located deep within the stomach mucosa, contiguous to the gastric glands. The production of histamine by these cells is elicited for the most part by the presence of the hormone gastrin and the neurotransmitter acetylcholine. Gastrin is produced almost completely by cells in the antral portion of the stomach mucosa in response to the presence of protein. Histamine is produced by parietal cells in intimate contact with hydrochloric acid and the amount of histamine produced by parietal cells is proportional to the amount of histamine produced by enterochromaffin cells. Acetylcholine is the end product of vagal nerve stimulation.

The hormone gastrin is produced in two different equally important forms, one a 34 amino acid polypeptide called G-34 and the other a 17 amino acid polypeptide referred to as G-17. The G-17 form is the more abundantly produced substance. Gastrin is produced by G or gastrin cells, which reside in the pyloric glands of the distal stomach. As protein enters the antral portion of the stomach, its presence directly evokes a stimulatory response from the gastrin cells of the pyloric glands, resulting in the release of gastrin into the lumen of the stomach. The mixing of the gastric luminal contents promotes the interaction of gastrin with the enterochromaffin cells

in the body of the stomach and histamine is released from the enterochromaffin cells. This histamine in turn stimulates the production of hydrochloric acid from parietal cells.

Pepsinogen production occurs in response to the presence of hydrochloric acid and acetylcholine. The hydrochloric acid most likely does not elicit its effect on pepsinogen production directly, but rather indirectly via interaction with the enteric nervous system and the initiation of enteric reflexes. Acetylcholine is produced as a result of the stimulation of the vagus nerves or the gastric enteric nervous plexus. So, while pepsinogen production appears to be essentially under nervous control, the rate of secretion of pepsinogen is significantly affected by the presence of hydrochloric acid in the stomach.

The gastric secretion of both hydrochloric acid and pepsinogen can be negatively modulated by the presence of excess acid. When the pH of gastric contents falls below that of 3.0, the gastrin mechanism for the stimulation of gastric secretion becomes nonfunctional, because the presence of hydrochloric acid seems to generate an inhibitory neural signal ceasing gastric secretion and directly blocking the secretion of gastrin by G cells. This acid-mediated feedback inhibition protects the stomach from the presence of excess acidity or too much pepsin, which could consequently damage the gastric mucosa. Similarly, this control mechanism also ensures that the pH of stomach contents lies within a range supportive of optimal enzymatic function. The optimal pH of stomach contents is considered to be approximately 3.0.

While the physical presence of material in the stomach generally stimulates the production of gastric secretions, this physical presence can also inhibit the production of the same during the gastric phase of gastric secretion. Indeed inhibitory hormones and the enterogastric reflex work together to reduce gastric motility at the same time as gastric secretions are reduced. The benefit of intestinal-mediated inhibition of gastric secretion is probably to slow the release of chyme from the stomach in situations where the small intestine is already filled or overly taxed in terms of function. The presence of irritating substances, hyper-osmotic fluid contents, fats, certain protein breakdown products, hypo-osmotic fluid contents or acid can stimulate the release of a number of different intestinal hormones. These hormones include, but are not limited to, gastric inhibitory peptide, somatostatin, vasoactive intestinal polypeptide, and secretin. The roles played by these hormones are relatively minor, except for that of secretin. Secretin is very important in the control of generation and release of pancreatic secretions, while at the same time reducing the amount of gastric secretions. The actual physical presence of food in the small intestine is capable of initiating a reverse enterogastric reflex that inhibits gastric secretion. This reflex is mediated through the vagus nerves, sympathetic nervous system, and myenteric nervous system and is elicited by a number of potential factors, which include distension of the bowel wall, the presence of mucosal irritation or the presence of certain protein digestive products or acid.

Gastric secretions in the stomach persist between the introduction of food loads, but at a very low level. The gastric secretions during these times are essentially that of mucus, with little pepsinogen and no acid being present. However, stress working via a mechanism similar to that described for the cephalic phase

can significantly increase the amount of gastric secretions and alter their character from that observed with baseline levels.

Within the proximal few centimeters of the duodenum is a large population of compound mucous glands, called Brunner's glands. These glands are located essentially between the pylorus and the entry points of the ducts from the gall bladder and pancreas. Brunner's glands produce large amounts of alkaline mucus, in response to the presence of secretin and various other gastrointestinal hormones, vagal stimulation, and direct stimulation. This alkaline mucus protects the sensitive intestinal mucosa from injury that could be caused by very acidic gastric secretions. The presence of copious amounts of bicarbonate ions in the mucus also helps neutralize the acid in the chyme entering the proximal small intestine. Sympathetic stimulation inhibits the secretion of mucus by the Brunner's glands.

The entire surface of the small intestine is covered with what appears to be stippling or small pits, which are openings to structures referred to as crypts of Lieberkühn. These crypts are interposed between intestinal villi and the mucosa covering both crypts and villi are populated with two visibly different types of cells, enterocytes and goblet cells. Enterocytes are present in large numbers and serve two different functions. Crypt enterocytes secrete copious quantities of water and electrolytes, but no enzymes. The stimulus for this secretion is not completely understood, but is thought to be largely based in local enteric nervous reflexes stimulated by direct contact of substances with the mucosa, gastrointestinal movement and the movement of gastrointestinal contents. There is some degree of hormonal regulation similar to that described for more proximal regions of the gastrointestinal tract and this control is for the most part due to the presence of the hormones cholecystokinin and secretin. Enterocytes located in the crypts can produce over 1.5 L of alkaline (pH 7.5–8.0) fluid per day. The purpose of the fluid is to act as a solvent for the end products of digestion, decrease the viscosity of the chyme, increase the fluidity of the chyme and improve surface contact of substances with the absorptive surface of the intestinal mucosa thereby enhancing absorption. Villous enterocytes, on the other hand, contain substantial quantities of digestive enzymes, which act to hydrolyze various food substances during the digestive process. These enzymes are located in the brush borders of the cells and include lactase, maltase, sucrase, isomaltase, intestinal lipase, and various peptidases. In addition to the absorption of the end products of digestion, villous enterocytes also absorb water and electrolytes. Goblet cells occur in moderate numbers and produce mucus for purposes of protection of the mucosa and lubrication. A very important aspect of the crypts of Lieberkühn is that cells in the deepest recesses of the crypts are continually undergoing mitosis. Newly produced cells migrate along the basement membrane away from the valley of the crypt to the tip of the villus. In this fashion, the cells covering each villus are being constantly replaced. As cells move along in an escalator-type fashion they mature in size and function, with the complete life cycle of an intestinal epithelial cell being approximately 5 days. Failure of this regenerative system or the inadequacy of it to compensate in a timely fashion for damage to intestinal epithelium can have substantial pathologic consequences to the organism. Enteroendocrine and Paneth cells are also found to be present deep in the intestinal glands. Paneth cells have not

been previously mentioned, but have the appearance of a protein-secreting zymogenic cell and secrete antimicrobial substances into the bottom area of the crypt.

Repeated reference has been made to the presence of various digestive enzymes in the gastrointestinal tract. Many of these enzymes come from the pancreas. When the pancreas is mentioned, the first thing that comes to mind is the hormone insulin. However, insulin is produced by a different population of cells in the pancreas than the digestive enzyme-producing cells. Furthermore, insulin is introduced into the blood and not the intestinal lumen. While the structure and function of the pancreas will not be addressed here, the nature of the secretory process and the secretions delivered to the intestine will be briefly discussed.

With proper stimulation, digestive enzymes are secreted and delivered in a copious amount and in an alkaline aqueous solution through two long ducts from the pancreas, the pancreatic duct and the accessory pancreatic duct, opening into the duodenum through the major and minor duodenal papillae, respectively. The main stimulus for pancreatic secretion is essentially the presence of chyme in the duodenum; however, similar to the stomach, the pancreas has three phases of secretion, cephalic, gastric, and intestinal. In the cephalic phase, the release of acetylcholine from nerve endings embedded in the pancreas is initiated by the same stimuli that promote gastric secretion. About 20% of a total postprandial bolus of enzymes is produced as a result of this initial stimulation, but little water and electrolytes are concurrently produced. This is because pancreatic enzymes are produced by compound acinous glands, which deliver their contents into acini and a system of ducts increasing in diameter, which eventually terminate in the confluence and formation of the pancreatic duct itself. The aqueous bicarbonate solution produced by the pancreas as the solvent for the protein component is secreted by the duct epithelium. In the absence of appropriate stimulation and an adequate supply of fluid, the enzymes remain stored in the acini and ducts until flushed into the duodenum with an adequate flow of water with its electrolytes and bicarbonate. In the gastric phase of pancreatic secretion, neural stimulation of the secretion of enzymes continues, adding about an additional 10% contribution to the total postprandial bolus production of enzymes. However, again as with the cephalic phase, there is little production of fluid to wash the protein material into the duodenum. As the stomach empties its contents into the duodenum, the presence of chyme initiates the release of the hormones secretin and cholecystokinin from the intestinal mucosa as well as acetylcholine from parasympathetic vagal nerve endings and other cholinergic nerves. The acetylcholine and cholecystokinin act together to stimulate even further enzyme production from appropriate acinar cells of the pancreas, with little stimulation of the bicarbonate-producing epithelial cells of the pancreas and its ducts. Secretin, while not stimulating the production of any enzymes, does stimulate pancreatic ductal epithelium to generate copious amounts of a watery and nonviscous bicarbonate solution. This alkaline solution contains bicarbonate at a concentration of approximately 145 milliequivalents per liter, very little chloride ion and almost no enzymes. Secretin is a 27 amino acid polypeptide produced by S-cells of the mucosa of the duodenum and jejunum in an inactive form called prosecretin. The prosecretin, upon being mixed with the intestinal luminal contents, where the pH is less than 5.0, is

converted into active secretin and absorbed into the blood. The critical reagent for this conversion of prosecretin to secretin is hydrochloric acid. It is important to note that secretin is only released from the mucosal epithelium of the small intestine when the pH of the intestinal contents falls below that of 5.0. Indeed, the production of secretin increases as the pH falls below that of 3.0. As the pancreatic juice mixes with the chyme, the hydrochloric acid that is present and the sodium bicarbonate that is added react to form sodium chloride and carbonic acid. The carbonic acid quickly breaks down to form carbon dioxide and water. The carbon dioxide is rapidly removed via absorption and transport in the blood to the lungs for discharge, leaving a neutral aqueous salt solution behind in the gut lumen. With this simple mechanism, acidity from the stomach is effectively controlled and additional peptic activity in the duodenum ceased. This is very significant because the mucosa of the small intestine cannot withstand the prolonged insult of gastric acid. Furthermore, the presence of bicarbonate in the pancreatic secretion causes an average pH of 8.0 of the secretion, which works to create an environment with a range of pH of 7.0–8.0, which is optimal for the highest degree of activity of pancreatic enzymes. Cholecystokinin, a 33 amino acid polypeptide is produced by I cells in the mucosal epithelium of the small intestine. The presence of long-chain fatty acids, peptones, proteoses, and to some degree hydrochloric acid initiates the secretion of this hormone, which moves via the blood to the pancreas. Upon reaching the pancreas, cholecystokinin causes the secretion of large quantities of digestive enzymes. Approximately 75% of the total postprandial bolus production of enzymes is produced as a result of the stimulation from cholecystokinin.

The total pancreatic secretion is capable of hydrolyzing fats, proteins, and carbohydrates and the character of the pancreatic secretion can be modified by the character of the chyme itself. Disaccharides and trisaccharides are products of the action of pancreatic amylase on such substrates as glycogen, starches, and most other carbohydrates. Neutral fats are reduced to fatty acids and monoglycerides under the action of pancreatic lipase. Pancreatic phospholipase and cholesterol esterase produce fatty acids and phospholipids and fatty acids and cholesterol, respectively. The most important and abundant pancreatic proteolytic enzyme is trypsin. Carboxypeptidase and chymotrypsin are also proteolytic enzymes of importance but are found to be present in lesser amounts than trypsin. Nucleases and elastases are also present in pancreatic secretions but are of lesser importance and in small amounts. Proteolytic enzymes produced by the pancreas are in enzymatically inactive forms, which are activated only upon entry into the gastrointestinal tract. This activation is achieved by the presence of kinases produced by the gastrointestinal mucosa and to a lesser degree by autocatalysis. The actions of nucleases and elastases are obvious. Trypsin and chymotrypsin cleave proteins and large polypeptides in such a fashion as to produce smaller peptides, but not individual amino acids. Carboxypeptidase, however, does hydrolyze peptides into individual amino acids.

Bile is another substance added to the intestinal stream to aid in the process of digestion. It is highly concentrated and primarily composed of a variety of different compounds classified as bile salts. These substances compose almost half of the total solutes found in bile, along with bilirubin, lecithin, cholesterol, sodium ions,

potassium ions, calcium ions, chloride ions, and bicarbonate ions. The cholesterol present is excess unused cholesterol and bilirubin, the principal breakdown product of hemoglobin. The secretion of bile by the liver is a two-part process. Hepatocytes contribute the bile salts, bilirubin, lecithin, and cholesterol to the actual bile secretion. Secretory epithelial cells lining the ductules and ducts of the biliary tree secrete water along with sodium and bicarbonate ions into the bile, which is injected into the duodenum after passage through a long duct, the bile duct, which opens into the duodenum through the major duodenal papilla. It should be noted that both the bile duct and pancreatic duct open into the duodenum through the major duodenal papilla. The aqueous component of bile can equal in volume that of the very concentrated organic component. Stimulated by the presence of fatty foods entering the duodenum, I cells in the mucosa of the upper small intestine release cholecystokinin, which acts as the main source of stimulation of contraction of the gall bladder, injecting bile into the intestinal lumen. A smaller contribution to this contraction comes from stimulation of the gall bladder by vagal nerves and the intestinal enteric nervous system. It is noteworthy that if no fat is preset in a meal, the gall bladder ejects very little of its contents into the duodenal lumen. The secretion of the alkaline aqueous and electrolyte component is stimulated by the presence of the hormone secretin. Up to approximately a half liter of bile is secreted every 12 hours and the gall bladder can only store maximally approximately 50 mL at any one time. Between a half liter and full liter of bile can be released into the small intestine each day, and in response to a fatty meal, the gall bladder can essentially completely empty itself with a period of about 60 minutes. The quantity of bile secreted each day by the liver is closely tied to the amount of bile salts that are available. As much as 2.5 g of bile salts can be trapped in enterohepatic circulation and if this amount increases, the production of bile increases. Indeed, if bile salts are exogenously administered, the daily production of bile will increase. This increase can be by as much as a factor of ten. As bile is stored in the gall bladder prior to its release into the small intestine, it is persistently being concentrated by the epithelium of the gall bladder. This concentration effect can range up to a factor of almost twenty-fold. While there are no enzymes present in bile, it nevertheless plays a prominent role in the digestion and absorption of fat. It accomplishes this through the presence of bile acids. These substances promote the emulsification of large fat droplets into tiny little particles that can be readily attacked by pancreatic lipases and aid in the absorption of the end products of fat digestion. As the digestive process initiates in the upper gastrointestinal tract, the gall bladder begins to empty its contents. This emptying is especially effective in response to a fatty meal and occurs within 20–30 minutes after a meal.

The epithelial cells of the mucosa of the large intestine do not secrete any enzymes, acid or factors into the lumen and are essentially of four different types, undifferentiated cells, immature absorptive cells and mucus-producing goblet cells. However, the predominant population is that of mucous cells, which produce the main secretion of the large intestine, mucus. Non-mucus-secreting cells located near the mucus-secreting cells produce bicarbonate ion, which gives the mucus of the large intestine an alkaline character. There are no villi in the large intestine as in the small intestine, but there are still many crypts of Lieberkühn, which are heavily populated with mucous cells.

Epithelial cells of the large intestine are replaced approximately every 6 days by the proliferation and differentiation of cells residing in the lower one-third of the crypt. Secretion in the large intestine is initiated or controlled by local nervous reflexes acting upon the mucus-secreting cells and direct stimulation of the mucus-secreting cells. Parasympathetic innervations arising from the pelvic nerves supply the distal half of the large intestine can significantly increase not only the amount of secretion of mucus, but also motility. The presence of mucus in the large intestine provides lubrication for the movement of the fecal stream, promotes the formation of the fecal mass, provides protection of the mucosa from the high level of microbial activity in the lumen, and as a result of its alkaline character (pH 8.0) provides protection of the mucosa from attack by acids in and formed in the feces.

We have talked about a number of different endocrine-cell secretions that occur along the digestive tract. It would seem appropriate that a summary of the geography and distribution of these types of secretions in the gastrointestinal tract be mentioned at the close of this section. Gastrin is present in the intestinal stream abruptly appearing at its highest levels in the pyloric antrum and persistent at that concentration to the proximal part of the duodenum, at which point the concentration of gastrin slowly falls to an almost immeasurable level by the end of the jejunum. Secretin is present in the intestinal stream abruptly appearing at its highest levels in the proximal duodenum and persistent at that concentration through the jejunum, at which point the concentration of gastrin slowly falls to an almost immeasurable level by the end of the ileum. Cholecystokinin is present in the intestinal stream in a pattern that essentially mirrors that of secretin. Glucagon-like substances, although not specifically discussed here, are found to be present in increasing amounts along the length of the entire gastrointestinal tract until the end of the jejunum, at which point the concentration of these substances reaches their zenith and then persists at that level until the end of the large intestine. Finally, somatostatin-like substances, although not discussed here, are also found to be present throughout the entire length of the gastrointestinal tract. These substances are found to abruptly start at high levels in the fundic portion of the stomach and persist at these levels until the proximal portion of the duodenum, at which point they rapidly fall to about half that of their initial levels. These reduced levels of somatostatin-like substances then persist through the remainder of the gastrointestinal tract. The principal enteroendocrine cells of the gastrointestinal tract, listed by cell type, location, hormone produced, and major action(s) are (1) A-cell, stomach, glucagon, hepatic glycogenolysis; (2) G-cell, pylorus, gastrin, stimulation of gastric acid secretion; (3) S-cell, small intestine, secretin, pancreatic and biliary bicarbonate and water secretion; (4) K-cell, gastric inhibitory polypeptide, inhibition of gastric acid secretion; (5) L-cell, glucagon-like substance, hepatic glycogenolysis; (6) I-cell, cholecystokinin, pancreatic enzyme secretion, and gall bladder contraction; (7) D-cell, somatostatin, local inhibition of other endocrine cells; (8) Mo-cell, motilin, increased gut motility; (9) EC-cell, serotonin and substance P, increased motility of gut; (10) D₁-cell, vasoactive intestinal polypeptide, increased gut motility, and ion and water secretion. Only the cells with major well-defined contributions to the function of the gastrointestinal tract have been discussed in this chapter; however, other cell types and their secretions have been mentioned as a matter of completeness.

DIGESTION AND ABSORPTION

Substances that enter the gastrointestinal tract include minerals, water, electrolytes, carbohydrates, vitamins, proteins, fats, and xenobiotics. Certainly, the vast majority of these substances fit into the general category of foods and nutrition. Compounds such as fats, proteins, and carbohydrates are macromolecules and of little nutritional value without digestion. To fully appreciate the function of the gastrointestinal tract, it is important to first understand the processes of normal digestion and absorption [18,21,25–27,42,48,51,62,64,82,84,95,96,108,114,128,131,139,142,143,167,169,185,197,217,225,229,277,283,284,291,299,300,312,349,351,370,376,381,382,389]. Certainly, xenobiotics are at least subject to the same biochemical processes of digestion and absorption as food and so an appreciation of the details of these activities as they pertain to food is important. Furthermore, xenobiotics have at least the potential to interfere with the normal processes of digestion and absorption, again making an understanding of the core processes important to predict and better understand toxic responses of the gastrointestinal tract. To put the process of absorption in perspective, each day the small intestine can absorb 50–100 g or more of carbohydrates, fat, amino acids, and ions in addition to about 7.5 L of water. However, this level of absorption constitutes only approximately 10%–20% of the total absorptive capacity of the small intestine. The large intestine can absorb even more water, but limited nutrients. This large excess of capacity for absorption speaks to the importance of absorption for sustenance of the organism. Vitamins and minerals have numerous and very specific mechanisms for uptake and these will not be discussed here.

There is one very important process that is a common thread of digestion and that is hydrolysis. While the enzymes that perform the hydrolysis change with the type of substrate, the basic reaction is the same and this reaction is the introduction of water into linkages that are specific for different types of substrates. The water is introduced not as molecular water, but rather as hydroxyl and hydrogen ions into a chemical bond which was previously formed in a condensation type of reaction in which water was extruded during the reaction in the form of hydroxyl and hydrogen ions.

The most common form of fat is the triglyceride or neutral fat and is composed of a molecule of glycerol and three fatty acids. Triglycerides are partly digested in the stomach via lingual lipase, which is secreted from glands in the mouth and then swallowed with saliva. The degree of digestion from this lipase is small and the overwhelming degree of digestion of fats occurs in the small intestine. The first step occurring in the small intestine is emulsification or the breaking down of the fat globules into small droplets. In this way, the relevant enzymes can operate on a greater amount of the fat per unit of volume and per unit of time since the surface area is increased. Indeed, as the droplets get smaller and smaller the surface area of a unit of fat can increase by a factor as large as a thousand. Gastric mixing contributes to the emulsification process, but the bulk of it occurs in the duodenum with the admixing of bile salts and lecithin, which interact with the fat globules. Lecithin is a phospholipid and its presence is critical to the process. The polar portions of lecithin and bile salts interact with water and enhance solubility. The nonpolar portions

of bile salts and lecithin are highly lipid-soluble and readily dissolve in fat. The fat-soluble portions of these hepatically-produced molecules dissolve in the surface layer of the fat globules, with the polar parts oriented in such a fashion as to orthogonally extend outward from the fat globule into the surrounding aqueous medium. This conglomeration or arrangement reduces the surface tension that normally exists at the interface between fat and water, facilitating emulsification and fragmentation of the fat globules. Lipases, which are water-soluble enzymes, can now more readily and effectively attack the fat. However, this attack can only take place on the surfaces of these tiny fat particles, emphasizing the importance of the process of emulsification. The most important intestinal enzyme for fat digestion is pancreatic lipase, which breaks triglycerides down into free fatty acids, diglycerides, and 2-monoglycerides. It should be mentioned that the accumulation of fatty acids and monoglycerides in the microenvironment of the digesting fat inhibits further digestion. Accordingly, it is essential that these materials be moved quickly away from the globule-associated digestive processes. Compounds known as bile salts serve this important function. Bile salts are produced by hepatocytes in an amount equal approximately to 600 mg per day. Dietary or endogenously produced cholesterol is the starting material for the synthesis or production of bile salts. In the first step, cholesterol is converted to chenodeoxycholic or cholic acids, which in turn combine with the amino acids glycine or taurine to form bile acids. As is evident, bile acids are nothing more than amino acid conjugates of the original acids, chenodeoxycholate or cholate. The sodium salts of these bile acids are the substances that are actually secreted into the bile. Bile acids and their salts can remove the fatty acids and monoglycerides away from the digesting globules of fat almost as quickly as the hydrolytic products are formed. A bile acid or salt is composed of a highly lipid-soluble sterol nucleus and a water-soluble polar group at the end of a hydrocarbon side chain. For reasons of thermodynamics, when in high concentration, bile acids and their salts aggregate together to form structures known as micelles. Micelles are collections of 20–40 molecules of bile acids/salts arranged in a spherical arrangement, with the central portion of the sphere being composed of sterol nuclei and the surface of the sphere being the polar groups on connecting hydrocarbon side chains. The micelles incorporate into their lipid cores the very tiny products resulting from fat emulsification and digestion and quickly and efficiently move these tiny fat droplets away from the sites of hydrolysis. Micelles are approximately 3–6 nanometers in diameter and because of the negative charges on the exterior polar groups; the micelle globule is readily dissolved in aqueous digestive fluid in stable fashion until absorption. These bile salt micelles also work to carry the free fatty acids and monoglycerides to the brush border of the intestinal epithelial cells, where they press deeply into the valleys of the undulating microvilli and are eventually absorbed into the blood.

Cholesterol occurs generally in the diet in the form of an ester, which is composed of one molecule of cholesterol and one molecule of fatty acid. Cholesterol ester hydrolase, which comes from the pancreas, hydrolyzes cholesterol esters into their components, a free fatty acid and cholesterol. Phospholipids also occur in the diet and are small molecules that are constructed of fatty acids and glycerol. However, only two of the alcohols of phospholipids are connected to fatty acid chains, with

the third being connected to a hydrophilic phosphate group. This phosphate group is in turn connected to a small hydrophilic compound like choline. Phospholipids can be hydrolyzed into a diglyceride (hydrolysis of carboxyl ester at position two of the glycerol) by the pancreatic enzyme phospholipase A₂. For both cholesterol esters and phospholipids, bile acid/salt micelles perform the same vital functions as for cholesterol in the processes of digestion and absorption.

Before leaving the topic of digestion and absorption of fats and bile acids and salts in particular, it should be mentioned that there is enterohepatic circulation of bile salts. Indeed approximately 95% of all bile acids and salts are reabsorbed into the circulation from the small intestine. The same bile compounds can recycle themselves approximately 18 times before eventually being eliminated from the body in the feces. This reabsorption is accomplished via an active transport type of process in the mucosa of the distal ileum and diffusion through the mucosa of the proximal part of the small intestine. Upon absorption into the blood, bile salts move via the portal blood back to the liver and into the hepatic venous sinusoids, where they are absorbed by hepatocytes and secreted again into the bile. Any bile compounds that are lost in the feces are replaced by the synthesis of new bile compounds.

The majority of carbohydrates that are ingested are mainly of three types, starches, sucrose and lactose. Starches are large, complex polysaccharides, while lactose and sucrose are disaccharides. Other types of carbohydrates that are ingested in much smaller amounts include cellulose, glycogen, amylose, dextrins, pectins, and insignificant amounts of various carbohydrate derivatives. Mastication initiates the process of digestion of carbohydrates with the introduction of ptyalin to a bolus of food. This enzyme is secreted by the parotid salivary glands and hydrolyzes starch into the disaccharide maltose and various other three to nine unit polymers of glucose. However, only a small amount of the total starch load will have been hydrolyzed by the time the food bolus has entered the stomach. After entering the stomach, this digestion will continue for approximately 1 hour, after which point the ingesta is thoroughly mixed with gastric secretions that have been added to the mixture. The acidity of the gastric secretions inactivates the salivary amylase, but by the time this happens approximately half of the available starch will have been hydrolyzed into maltose. Hydrolysis of carbohydrates continues in the small intestine with the introduction of pancreatic secretions. Pancreatic amylase performs the same activity as oral amylase, but has a much higher throughput or turnover number. Indeed, almost all remaining starches are converted to maltose and other very small polymers of glucose in the time period between entering and leaving the duodenum. The small polymers of glucose and the disaccharides produced as a result of prior hydrolytic cleavage need to be further reduced to monosaccharides for intestinal absorption to occur. Four enzymes critical to achieving these chemical breakdowns are sucrase, maltase, alpha-dextrinase, and lactase. These enzymes are found in the enterocytes lining the microvilli brush border of the small intestine. As materials come in contact with these enzymes, further hydrolysis takes place. Lactose is split into a molecule of glucose and one of galactose; sucrose is split into a molecule of glucose and a molecule of fructose. Maltose and other glucose polymers are reduced to single units of glucose. The final hydrolytic product of carbohydrate digestion is a

monosaccharide and most commonly glucose. These terminal products are all very water-soluble and readily absorbed.

Proteins are macromolecules which are polymers of amino acids joined together via peptide linkages. These linkages are also broken down via hydrolysis by means of the enzyme pepsin to produce compounds like peptones, polypeptides, and proteoses. Pepsin can also hydrolyze collagen, a significant component of meat and which is very resistant to degradation by a number of other enzymes. If collagen itself is not hydrolyzed, the effectiveness of hydrolysis or digestion of protein in materials like meat is highly impaired and hence so is absorption. The products resulting from the breakdown of protein by pepsin enter the small intestine, the chief site for the digestion of protein. As soon as stomach contents are moved into the duodenum, the chyme is attacked by several pancreatic enzymes, including proelastase, chymotrypsin, carboxypolypeptidase, and chymotrypsin. Proelastase is converted into elastase which degrades elastin. Trypsin and chymotrypsin degrade protein into polypeptide fragments of varying size. Carboxypolypeptidase trims amino acids off of the carboxy termini of the various polypeptides products produced by previous enzymatic cleavage. Unlike carbohydrates, only a small component of protein is completely hydrolyzed to component amino acids by pancreatic enzymes. Indeed, protein for the most part is reduced only to dipeptides, tripeptides, tetra peptides, etc.

The total amount of fluid that must be absorbed by the intestines on a daily basis is approximately 9 L and this volume is comprised of up to 1.5 L of ingested fluid and 7.5 L of secreted fluid. Approximately 80%–90% of this fluid is absorbed in the small intestine, leaving the remainder to be absorbed in the large intestine. The stomach lining lacks a villus absorptive surface and has very tightly woven junctions between apposed epithelial cells, making the gastric mucosa a poor area for absorption. Exceptions to this are highly lipid-soluble materials, salicylates, and ethanol, which can all be directly absorbed through the stomach wall in small quantities.

The mucosa of the small intestine has covering its entire surface basically from the point at which the common bile duct empties into the duodenum to the terminus of the ileum millions and millions of very small villi, which project approximately 1 mm from the surface of the mucosa. While the villi are somewhat less populous near the end of the small intestine, they generally occur in such a density that they exist in very close apposition to one another. Their presence increases the surface available for absorption by a factor of almost ten. In addition to this gross anatomic adaptation of the gut, which acts to increase and facilitate absorption, a microscopic adaptation increases the surface available for absorption by a factor of almost twenty. This feature is the brush border, which is found on the free surface of each intestinal epithelial mucosal cell on each villus. The brush border is multiple invaginations or evaginations of the enterocytic membrane, which result in the formation of microvilli. There can be as many as 1000 microvilli on the free surface of each intestinal enterocyte. These microvilli are approximately 0.10 μm in diameter and 1 μm in length. Collectively these adaptations work together to enhance the intestinal surface area available for absorption by a factor of almost 500.

The basic mechanisms of absorption include diffusion, active transport, and solvent drag. There are two different kinds of diffusion, simple and facilitated.

In simple diffusion, kinetic molecular motion moves molecules or ions through a membrane, pore, gate, opening or any intermolecular space without the necessity of binding with a carrier protein in the membrane. In this case, the rate of diffusion is dependent on the concentration of the substance, the kinetic energy of the substance and the number and size of openings through which the substance can pass. Facilitated diffusion requires the participation or help of an ancillary or carrier protein. This carrier protein chemically binds with the substance being moved and shuttles it through a membrane. Active transport refers to the process of moving or transporting materials against a concentration gradient. It is important to appreciate that active transport energy is imparted to the substance being transported. There are two kinds of active transport, primary and secondary, both of which utilize carrier proteins that span or access both sides of a membrane. For primary active transport, the process energy is derived directly from the breakdown of adenosine triphosphate or an alternate high energy phosphate bond compound. However, for secondary active transport, the energy for the process comes from energy stored in the form of ionic concentration differentials between two sides of a membrane, this difference being created by the process of active transport. In the case of solvent drag, dissolved substances can be pulled along as a solvent is absorbed because of various physico-chemical forces that exist between the solvent and solute.

Transport systems exist not just in the surface membranes of cells, but also intracellular membranes and intercellular membranes or junctions. It should be appreciated that transport through the intestinal epithelium must occur through a sheet of cells and not just simply through one cell membrane. The basic mechanism for the transport of a substance through a cellular sheet is first active transport of the substance through the cell membrane on one side of a cell followed by simple or facilitated diffusion through the cell membrane on the opposite side of the cell. A variety of different combinations and locations of transport systems exist throughout the gut for the absorption process, but the basic principles remain the same, regardless of the nature of the substance involved.

For completeness, it should be mentioned that in addition to the above processes a very small contribution is made to absorption by the physical process of pinocytosis. Pinocytosis occurs continually in most cells, but at widely differing rates according to cell type. This process is the means by which most macromolecules and proteins enter cells; however, the process can involve large carbohydrates, lipids, and other substances. Indeed the degree of pinocytosis significantly increases as macromolecules attach themselves to a cell membrane. Particles of several thousand nanometers in diameter have been reported to have been taken up by the duodenum. Latex particles of 22 μm in diameter have been carried through the cytoplasm of intestinal epithelium and deposited into the lamina propria, with subsequent absorption into the lymphatics. For this process to occur, proteins bind to specialized receptors that are embedded in a cell membrane and that are specific to that type of protein or sequence within a protein. These receptors are located in miniscule depressions on the cell's surface called coated pits. Deep to the coated pit and on the interior surface of the cell membrane is a net-like array of the protein clathrin and other proteins possibly including actin and myosin. When the binding between protein and

receptor is adequate and complete, the pit invaginates, enveloping the entire protein and a small amount of extracellular fluid. At the completion of this invagination process, the invaginated portion of the cell membrane pinches off in such a fashion as to maintain cell membrane integrity and form a totally sealed vesicle, called a pinocytotic vesicle, which is inside the cell. The pinocytotic vesicle now combines with a lysosome and is digested or broken down by the machinery of the cell. Small degradation products such as sugars, amino acids, etc. can diffuse through the membrane of the digestive vesicle into the cytoplasm, where they can be immediately utilized or follow the paths of similar substances that are being absorbed via other mechanisms. Undigested residual material is excreted directly through the cell membrane in a process known as exocytosis.

Each intestinal villus is almost finger-like in shape and if a cross-section is transversely cut at mid level through a villus, it would appear somewhat like a small onion. The outermost layer would be the brush border, as discussed previously. Immediately deep to this would be the enterocytes themselves firmly anchored to the deeply underlying basement membrane. The inner area of each villus is a network of vasculature embedded in loose connective tissue. Capillaries connect a supplying artery with a draining vein. At the innermost central core is the very important central lacteal, which is located approximately along the central axis of each villus and collects the lymphatic fluid draining the villus. The lymphatic system is an accessory route by which fluid can flow from interstitial areas eventually back into the blood. However, the lymphatic fluid can also carry whole proteins, large molecules, fat and particulate matter away from a local tissue space eventually back into the blood. This alternative route compensates for the fact that none of these materials can be directly absorbed into the bloodstream.

Water is absorbed from the small intestine by diffusion obeying the laws of osmosis. However, water can move in two different directions. It can flow from the fluid intestinal contents into the villous blood or from the plasma of the blood into the lumen of the small intestine. The latter can occur when the chyme hyperosmolar in character.

A variety of ions are absorbed from the small intestine and they include but are not limited to sodium, calcium, potassium, magnesium, iron, chloride, bicarbonate, and phosphate. Between what is secreted into the intestinal lumen and what is absorbed from the intestinal contents a significant deficit exists between the influx and efflux of sodium. This differential strongly favors the loss of sodium. Accordingly, under normal conditions with no intervening pathology or toxicology, an amount of sodium equal to about 10%–20% of the total body sodium must be absorbed each day. Fortunately, sodium is rapidly absorbed through the mucosal epithelium of the intestine. Sodium ion concentration in intestinal contents averages approximately 142 milliequivalents per liter, which approximates that of plasma, but intracellular sodium ion concentration is only approximately 50 milliequivalents per liter. Consequently, sodium follows the concentration gradient and moves by facilitated diffusion from the liquid intestinal contents through the brush border of the epithelium of the small intestine into the cytoplasm of the enterocytes. This sodium is then in turn moved from the cell interior through the baso-lateral aspect of the

cell (base of cell rests on basement membrane, brush border side of cell is exactly opposite and lies in contact with the chyme) by means of active transport and the energy of adenosine triphosphate into a paracellular space. Paracellular spaces are small areas located between adjacent enterocytes and bound deeply by the basement membrane and superficially by the tight junctions welding adjacent intestinal epithelial cells together at points on the lateral walls just beneath the brush border. Some chloride ion can be absorbed simultaneously with sodium ion, as the opposing electrical charges work in an attractive fashion causing a drag effect. Additional sodium is also absorbed as a consequence of the necessary and required activity of the sodium-potassium pump. As the sodium ions accumulate in the paracellular spaces, water moves by osmosis into these same areas. The water for the most part enters these paracellular spaces through the tight junctions mentioned earlier, but a small portion does arise from or through the cell membranes of the enterocytes themselves. As the water flows into and through the paracellular spaces, the ions and water eventually enter the blood circulating in the villus. Before leaving the topic of sodium ion absorption it should be mentioned, that under certain physiologic conditions, the adrenal cortical hormone aldosterone can be released and this hormone can significantly enhance the absorption of sodium by the intestinal epithelium.

Chloride absorption occurs rapidly in the duodenum and jejunum and also occurs via diffusion. As sodium ions follow the movement as described previously, an electric potential is established, with the intestinal contents becoming more negative. Chloride ions follow the electrical gradient trailing the sodium ions and moving from the more negative area to the more positive area. Chloride ions are absorbed by enterocytes located on the villi of the ileum and large intestine in a process which secretes a bicarbonate ion for each chloride ion absorbed. The purpose of this activity is most likely to provide some degree of basification of the acidic products present in the intestinal stream intestinal contents as well as to maintain the electrochemical gradient.

Bicarbonate ions are present in large amount in the small intestine not only as a result of intestinal secretory activity but also as a result of secretions from the liver and pancreas. It is important to recapture this ion to maintain a normal acid-base homeostasis in the body. The reabsorption occurs somewhat indirectly in the duodenum and jejunum. Bicarbonate ion combines with existing hydrogen ion to form carbonic acid. This acid is relatively unstable and decomposes rapidly to form carbon dioxide and water. The water remains as part of the intestinal contents and can follow the absorptive path described previously. The carbon dioxide is very soluble, readily absorbed and quickly moved to the blood in the villi. Carbon dioxide is eventually cleared from the body through the lungs with expiration.

In general it is fair to say that monovalent ions are absorbed from the lumen of the gut with great ease, but bivalent ions are absorbed in only small amounts. Indeed the maximal absorption of bivalent ions can be as little as only two percent of the maximal absorption of monovalent ions. That said potassium (monovalent), magnesium, and phosphate are most likely actively absorbed through the intestinal mucosa. Calcium absorption is very highly regulated and is absorbed heavily from the duodenum. Other factors controlling calcium absorption are the presence of Vitamin D, parathyroid hormone, and the degree of physical activity of the organism (Wolf's Law).

Nutrients that are absorbed fall into three general classes, proteins, fats, and carbohydrates, the same general classes as for digestion. For the most part, carbohydrates are ultimately absorbed as monosaccharides and the most common monosaccharide is glucose. The sugar monomers of fructose and galactose are also absorbed, but they exist in smaller amounts. Absorption of glucose is via an active transport process. Disaccharides are absorbed in only small amounts and sugar polymers of greater numbers are virtually not absorbed at all.

A fact that is not commonly appreciated is that in the absence of sodium ion, no glucose absorption can occur. This is because glucose is co-transported with sodium ion.

We mentioned earlier that sodium follows a concentration gradient and moves by facilitated diffusion from the liquid intestinal contents through the brush border of the mucosal epithelium of the small intestine into the cytoplasm of the enterocytes. This sodium is then in turn moved from the cell interior through the baso-lateral aspect of the cell by means of active transport into the paracellular space. The facilitated diffusion is accomplished with the aid of a transport protein, but sodium ion will not be transported into the interior of the cell unless the transport protein combines with another appropriate moiety, glucose. In this process, both glucose and sodium are co-transported into the interior of the enterocyte. It is important to appreciate that the low concentration of intracellular sodium ion is the energy driving this process. The glucose, once inside the enterocyte is moved outside the cell through the basolateral membrane of the intestinal epithelial cell into the paracellular space by means of facilitated diffusion. Once in this space, the glucose is readily taken up by the blood circulation of the villus. Galactose is absorbed by the same exact mechanism as glucose; however, fructose is not. Fructose does move through the enterocyte by means of facilitated diffusion, but the movement is not coupled with sodium ion transport. As fructose enters the cell, most of it is phosphorylated, converted to glucose and then transported as glucose into the paracellular space. This lack of co-transportation with sodium ion leads to a significantly reduced rate of transport when compared to that of glucose.

Most proteins are absorbed as dipeptides, tripeptides, and amino acids directly through the enterocytic membranes that line the lumen of the gut, chiefly in the areas of the duodenum and jejunum. Here again a sodium ion co-transport mechanism works in the same fashion as that for sodium and glucose. The enterocytes have a brush border that is composed of hundreds of microvilli projecting from the surface of each cell. Anchored in the membrane of each of these microvilli are peptidases that extend into the milieu of the intestinal luminal space. There are two classes of peptidases that are worthy of note, dipeptidases and aminopolypeptidases. These enzymes degrade any large polypeptides into dipeptides, tripeptides, and amino acids. The small peptides or amino acids then bind to a specific transport protein in the enterocyte's microvillus membrane that requires sodium ion binding as a prerequisite to transport. The amino acids, dipeptides, and tripeptides are readily transported through the membrane of the microvilli into the interior of the enterocytes. As the sodium ion moves down the electrochemical gradient into the enterocyte, it pulls along with itself an amino acid or small peptide. Some amino acids do not require a sodium ion co-transportation mechanism, but rather move via facilitated

diffusion and specialized membrane transport proteins. While much still remains to be elucidated with regard to the absorption of amino acids and small peptides, it is known that there are at least five different types of amino acid and peptide transport proteins in the luminal or free surface membrane of enterocytes, reflective of the different chemical characteristics of different amino acids. When in the enterocytic cytosol, additional peptidases, which are specific for different types of amino acids, act to degrade the small polymers of amino acids into individual amino acid components. This process occurs within seconds to minutes and the single amino acids are moved from the enterocyte most likely via facilitated diffusion or active transport processes to enter the villus blood. It can be seen from this that ultimately, almost all of the digestion products of protein that are absorbed are taken up in the form of amino acids. Only rarely are peptides and whole proteins actually absorbed into the blood. Indeed, the absorption of proteinaceous material can result in the development of serious allergic or immunologic disorders.

Because of their lipid solubility, the monoglyceride and fatty acid end products of fat digestion diffuse readily from their transporting micelles through the membrane of the enterocyte into the interior of the cell, when contact is made between the micelle and the microvillus membrane. The unloaded micelles remain in the chyme, where they accumulate more monoglycerides and fatty acids to repeat again and again this ferrying or transport cycle until fat absorption is complete. The importance of micelles to this whole process is illustrated by the fact that in their presence almost 100% of the fat in any food load is absorbed, but in the absence of micelles, only about 50% of the fat in a particular food load is absorbed. When the monoglycerides and fatty acids are inside the enterocyte, they are taken up by the endoplasmic reticulum, where they are recycled to form new triglycerides that are extruded into the lymph. These triglycerides then become part of lymph chylomicrons, small structures which transport triglycerides through the lymphatic system to a point at which they are eventually mixed in with the blood of the circulatory system. It should be noted that some proportion of very short, short, and medium-chain length fatty acids can be absorbed directly into the portal venous blood supply of the villi rather than following the processing path and journey through the lacteals and lymphatic system as just described. The reason for this difference is probably because of the greater degree of water solubility of these types of compounds.

The large intestine is the major site for the absorption of water and electrolytes in the gastrointestinal tract and can absorb up to a maximum of approximately 5–8 L of fluid containing electrolytes daily. However, if this maximum capacity is exceeded, watery stool or diarrhea can result with the loss of water and electrolytes. Similar to the small intestine, the mucosa of the large intestine has a large capacity for absorption of both sodium and chloride ions. Indeed, by the time large intestinal contents are ready for excretion, only a couple of milliequivalents each of sodium and chloride ions are found to remain in the feces. The principles of absorption for ions and water are the same for both the small intestine and large intestine, with one important difference. The tight junctions that exist between the epithelial cells are much tighter in the large intestine than in the small intestine. This closer apposition establishes a much stronger barrier, because any diffusion back into the intestinal lumen

is prohibited. This increases the extent of absorption of ions, especially sodium and chloride, and establishes a greater concentration gradient of ions. When the hormone aldosterone is being secreted, the absorption process becomes even more stringent or efficient. Similarly, as in the small intestine, the mucosa of the large intestine secretes bicarbonate ion into the intestinal lumen to counteract the acidity of microbial end products produced by bacterial fermentation. As bicarbonate ions are secreted by the mucosa of the large intestine, equal milliequivalent amounts of chloride ion are absorbed via the same mechanism as described previously. As observed more proximally in the gastrointestinal tract, both sodium and chloride ions exist in osmotic and electrochemical gradients across the barrier of the epithelial mucosa of the large intestine and the energy of these gradients indirectly drives the absorption of water from the intestinal fecal stream.

Unless there is a direct-acting pharmacologic or toxic effect, xenobiotics present in the gastrointestinal tract do not produce effects until they are absorbed into the system [2,195,333,334,371,372,374,396]. Agents can be absorbed anywhere along the gastrointestinal tract via any mechanism that has been described. These mechanisms include, but are not limited to passive diffusion, facilitated diffusion, active transport, and co-transport. The specific details with regard to each of these mechanisms have already been addressed previously in this text; still there are a number of factors that need to be appreciated with regard to the absorption of xenobiotics from the gastrointestinal tract, regardless of the method of administration or introduction. The composition of diet, such as fat content, can produce a significant effect on the absorption of individual xenobiotics and an understanding of these potential effects needs to be achieved for any test article. Similarly, fasting can also affect the profile of absorption. Specialized transport systems do commonly exist in the gastrointestinal system, and it is not rare that a xenobiotic could be absorbed solely or partly via one of these types of systems. While much still remains to be learned with regard to these systems, over the last few years a greater understanding of these specialized transport systems (specialized active transport systems) has developed and it is now thought that there are a number of different families of xenobiotic transporters. One of these families is the multidrug resistant proteins (mdr) or p-glycoproteins. This transporter moves chemotherapeutic drugs out of neoplastic cells and thereby contributes to the development of neoplastic resistance. Additionally, the mdr transporter system is responsible for the movement of chemicals out of hepatocytes, renal epithelial cells, intestinal epithelial cells, brain endothelial cells and protection of the fetus. The multi-resistant drug proteins (mdp) are also responsible for the movement of xenobiotics out of cells, but favor phase II conjugates like glucuronides and glutathione adducts as substrates. Acids, bases and neutral compounds can be transported by organic-anion transporting peptides (oatp), which also play a prominent role in the hepatic absorption of xenobiotics. The organic anion transporter (oat) group is very important in the uptake of anions, especially in the kidney. The organic cation transporter plays a significant role in the absorption of xenobiotics and is of special importance in renal and liver cells. Metals, nucleotides, and di- and tri-peptides are absorbed with the assistance of the divalent-metal ion transporter (dmt), nucleotide transporter (nt), and peptide transporter (pept), respectively. Thallium, cobalt, and manganese are absorbed by

the same system that absorbs iron and lead are absorbed by the calcium uptake system. Congeners of various pyrimidines can be transported by pyrimidine transport systems. Co-transport systems exist also, but are most common for metals and metal-containing compounds. In such systems, the presence of one moiety can affect the absorption of another in a positive or negative fashion. Despite the broad spectrum of systems available for absorption, it is important to emphasize that the number of xenobiotics that are *actively* absorbed or absorbed via co-transportation by the gastrointestinal tract is low and that most xenobiotics enter the body via the process of simple diffusion across the gastrointestinal mucosal epithelial barrier.

If one looks at the locations of these specialized transport systems in the small intestine and colon, the following general observations can be made. The specialized transport of calcium, iron, fatty acids, and chloride is at a high level in the proximal small intestine, moderate level in the mid-small intestine, low in the terminal small intestine and nonexistent in the colon. Bile salts and Vitamin B₁₂ are transported only to a small degree in the mid portion of the small intestine and a large degree in the terminal small intestine. Sodium is actively transported at a high level throughout pretty much the entire length of gastrointestinal tract. Pyrimidines are transported only in small amounts and only in the proximal and mid portions of the small intestine. Sugars and neutral amino acids are transported in moderate to high amounts through the entire length of small intestine, but not in the large intestine. Basic amino acids and triglycerides are transported in moderate amounts pretty much through the entire length of the small intestine, but not in the large intestine. Gamma globulins are transported in increasing amounts through the length of the small intestine only, reaching their zenith of absorption in the terminal ileum. Hydrogen ion transport occurs at low levels in the middle portion of the small intestine and moderate levels in the distal portion of the small intestine and the entire large intestine.

Generally speaking if a test article has a molecular weight below that of ten thousand, at least some degree of absorption will occur [1,20,40,71,79,107,119,127,135,153,192,281,293,309,317,319,345,346,373,380,399,401,412,413,419]. How much absorption takes place will depend in part upon the physical-chemical properties of the molecule (e.g., solubility, octanol: water partition coefficient, dissolution rate, etc.) as well as the characteristics of the formulation in which the xenobiotic is delivered. If xenobiotics are delivered as large particles, absorption can be very low. Similarly, insoluble solids have little opportunity to make contact with the intestinal mucosa. Animal physiology can also have a profound effect on absorption. It is not uncommonly assumed that the gastrointestinal absorption of xenobiotics is similar or even identical across species, but this is not the case and numerous studies have been performed which demonstrate significant differences in bioavailabilities between species that are supported with adequate distribution, metabolism, elimination, and excretion data. For example, it has been suggested that the rate limiting step in the absorption of any xenobiotic is the removal of that xenobiotic from the very thin, static or non-agitated water layer, which lies in intimate contact with the lipophilic membranes of the epithelium of the gastrointestinal mucosa. Species differences in the absorption of xenobiotics may well be due to differences in the thickness of this water layer. Differences of size, anatomy, histology, and histophysiology

between the gastrointestinal tracts may also contribute in a profound fashion to differences in the absorption of xenobiotics across species. Considerations of size alone may be of paramount importance, considering the fact that as mentioned previously most xenobiotics are absorbed via the process of passive diffusion. To that point, gastrointestinal absorption is highly dependent upon the values of local pH within the gastrointestinal tract, and here again important differences between species have been found to exist. Neonates have a poorly developed intestinal mucosal barrier, permitting facile absorption of a wide variety of substances. Inflammation and parasitic infestation can also lead to enhanced or altered absorption. Scarring and thickening of the intestinal wall can lead to decreased absorption. It is important to remember that digestive fluids, microbial flora and other moieties in the gastrointestinal stream may modify the structure of a chemical or bind in some fashion to it and thereby radically change the absorptive profile of the test article as well as its anticipated pharmacology and toxicology. Secondary amines can be converted under the right circumstances into carcinogenic nitrosamines. An interesting observation is that the dilution of a xenobiotic typically results in an increased rate and degree of absorption. Whether this increase in absorption is a direct result of dilution or merely a consequence of increased dose volume, more rapid emptying of the stomach and the increased transit of material into the small intestine, where the absorptive surface area is greater is not completely known. It bears repeating that one of the most important aspects of the absorption of xenobiotics from the gastrointestinal tract is the fact that lipid-soluble compounds are absorbed more readily than water-soluble compounds. This is because the former are in a nonionized state and the latter are in an ionized state. Two general rules burgeon from this concept. The first is that weak organic acids exist in the nonionized and lipid-soluble form in the stomach. Accordingly one might expect significant absorption of weak organic acids to take place in the stomach, but as a result of differences in the characteristics of gastric mucosa versus that of the intestinal mucosa, increased residence time in the intestine and exposure of the weak organic acid to significantly greater surface area in the intestine, the greatest degree of absorption of weak organic acids takes place in the intestine. This is because blood flow rate and absorptive surface area are more important than pH for the absorption of weak acids. The second is that weak organic bases exist in a nonionized and lipid-soluble form in the intestine and as a direct result of this are indeed absorbed in the intestine. It was mentioned previously that the lipid-soluble or more lipid-soluble form of any substance is the preferred form for absorption and ethylenediaminetetraacetic acid (EDTA) is thought to increase the absorption of some compounds by improving lipophilicity and thereby enhancing intestinal permeability. However, there is apparently an upper limit to the degree of lipid solubility, as some exceedingly lipid-soluble compounds do not dissolve in the gastrointestinal contents and their absorption can be extremely low. Not surprisingly, organic acids and bases of small molecular weight are typically absorbed by simple diffusion. Remember that gastric juices are acidic and intestinal contents are essentially neutral to very slightly basic and the degree of lipid solubility of weak organic acids or bases varies with the proportions of populations of ionized and nonionized forms of the xenobiotic. The proportions of these populations can differ markedly

within different regions of the gastrointestinal tract, because of the changes in the pH of the milieu. Approximate average values of pH in different regions of the canine gastrointestinal tract have been reported by Davies and Morris to be: anterior stomach, pH 5.5; posterior stomach, pH 3.4; proximal small intestine, pH 6.2; terminal small intestine, pH 7.5; cecum, pH 6.4; colon or large intestine, pH 6.5; and feces, 6.2. So, for compounds that are organic in nature and either acidic or basic, the relative proportion of ionized and nonionized species is of paramount importance. This is best appreciated with an understanding of the Henderson-Hasselbach equation, which states that for a weak acid

$$\text{pKa} - \text{pH} = \log \left\{ \frac{[\text{nonionized}]}{[\text{ionized}]} \right\}$$

for a weak base,

$$\text{pKa} - \text{pH} = \log \left\{ \frac{[\text{ionized}]}{[\text{nonionized}]} \right\}$$

A derivation of this equation is not appropriate for this text, but can be found in any biochemistry textbook. However, some examples in the application of this equation might be useful.

For the case of a weak base having a $\text{pKa} = 5$ and residing in the stomach at a gastric environmental $\text{pH} = 2$,

$$\begin{aligned} \text{pKa} - \text{pH} &= \log \left\{ \frac{[\text{ionized}]}{[\text{nonionized}]} \right\} \\ 5 - 2 &= \log \left\{ \frac{[\text{ionized}]}{[\text{nonionized}]} \right\} \\ 3 &= \log \left\{ \frac{[\text{ionized}]}{[\text{nonionized}]} \right\} \\ 10^3 &= \frac{[\text{ionized}]}{[\text{nonionized}]} \\ 1000 &= \frac{[\text{ionized}]}{[\text{nonionized}]} \end{aligned}$$

In this case the equilibrium does not favor absorption.

For the case of a weak acid having a $\text{pKa} = 4$ and residing in the intestine at an intestinal environmental $\text{pH} = 6$,

$$\begin{aligned} \text{pKa} - \text{pH} &= \log \left\{ \frac{[\text{nonionized}]}{[\text{ionized}]} \right\} \\ 4 - 6 &= \log \left\{ \frac{[\text{nonionized}]}{[\text{ionized}]} \right\} \\ -2 &= \log \left\{ \frac{[\text{nonionized}]}{[\text{ionized}]} \right\} \\ 10^{-2} &= \frac{[\text{nonionized}]}{[\text{ionized}]} \\ 1/100 &= \frac{[\text{nonionized}]}{[\text{ionized}]} \end{aligned}$$

In this case the equilibrium does not favor absorption.

To this point we have focused on the absorptive capabilities of the gastrointestinal tract and tacitly assumed the presence of no other confounding processes. However, it is important to point out that the gastrointestinal tract is not just a flexible tube capable of absorption, but is a metabolically active organ. The flow of blood from and to the gastrointestinal tract not only serves as a vehicle for the transport of substances to the body as a whole, but also as a means to provide the delivery of vital nutrients to the gastrointestinal tract itself for the performance of all of its functions. Using man as an example, the gastrointestinal tract devoid of contents comprises approximately 2% of the total body weight of an average human and is very well perfused by the circulation system. In fact, the human gastrointestinal tract as defined in this chapter (esophagus, stomach, small intestine, large intestine) receives approximately 20% of the total cardiac output and has a blood flow in a resting normal subject of about 1050 mL/min. To put this into perspective, this amount of blood flow is greater than that of the brain or skeletal muscle and about equal to that of the liver and kidney. The metabolic activities of the liver and kidney are well appreciated and so the potential for metabolism in the gastrointestinal tract should not be ignored. It should be emphasized that the above figures are provided for purposes of relative comparison only and should not be taken as exact values for the canine gastrointestinal tract. This potential for metabolism leads us to at least mention the “first-pass effect.” Under most circumstances, substances are absorbed into the body system via mechanisms and routes previously described. However, for some xenobiotics a “first-pass effect” can be quite significant. In this process, xenobiotics are removed in normal fashion from the intestinal stream, but then either metabolized by intestinal epithelial cells directly or hepatocytes after delivery to the liver by the portal blood supply, which drains the intestinal bed. The important aspect of this process is that parent compound can be significantly reduced in amount by metabolism before it even enters the systemic circulation. Indeed, this effect can be quite substantial for some compounds and in some cases can essentially obliterate the total amount of parent compound administered. With regard to the metabolic transformation of xenobiotics, the intestines are considered to have a moderate level of metabolic activity, equal to that of the kidney and second only in amount to that found in the liver. While a variety of phase I enzymes can be found in the gastrointestinal tract, the most common phase I enzymes found in the canine small intestine are from the CYP2C subfamily. The most phase II reactions that take place in the canine intestine are glucuronidation, ethereal sulfation, methylation, acetylation, and sulfation. While the first-pass effect may be a formidable protection mechanism, it at the same time can present a substantial barrier to pharmacotherapy. The biotransformation of xenobiotics is addressed in more detail elsewhere in this reference.

In summary, it is fair to state that much has been learned about the principles of gastrointestinal absorption of xenobiotics from the mechanisms of absorption of nutrients, carbohydrates, proteins, and fats. Xenobiotics penetrate the gastrointestinal epithelium to a degree and facility that is related to the chemical structure of the substance and its physico-chemical properties. The gastrointestinal absorption of xenobiotics occurs mostly by diffusion and is highly dependent upon the values of

local pH within the gastrointestinal tract. A very important physico-chemical property of a xenobiotic is its pK_a , which governs how much of a compound will exist in a lipophilic, nonionized, and absorptive form versus a hydrophilic, ionized, and nonabsorptive form at a specific pH. Keep in mind that a difference of only a single unit of pH can translate into a change of concentration of ionized versus nonionized species of one order of magnitude.

The discussion of absorption to this point has focused on the movement and transport of nutrients and xenobiotics into the system. However, what about the movement of xenobiotics out of the system? It is commonly assumed that the presence of xenobiotics, whether in parent form or not, in the feces is the result of either lack of absorption or excretion into the bile. While this is true for many compounds, it is not true for all. Indeed, for some substances, there appears to be a direct transfer from the blood through the gastrointestinal mucosal epithelium into the lumen of the gastrointestinal tract. Again passive diffusion is probably the most common mechanism for this transfer, but cellular exfoliation may well be another mechanism for the elimination of some xenobiotics into the intestinal stream. The exfoliation of gastrointestinal epithelial cells into the gastrointestinal lumen or intestinal excretion is a slow process. Accordingly, this mode is only a pathway of significance for xenobiotics that exhibit low rates of biotransformation or low rates of hepatic (biliary) or renal clearance. Not surprisingly, it has been found that as the lipophilicity of gastrointestinal contents increases, the rate of intestinal excretion increases.

Other ways in which xenobiotics can enter the gastrointestinal tract and be excreted in the feces are the ingestion of secretions that contain the xenobiotic. Such secretions would include those that move up the tracheobronchial tree and are swallowed, secretions from salivary glands and secretions from the stomach, intestines, liver or pancreas. With the exception of the liver, these contributions are of generally small amounts and of little concern; still the potential for the recirculation of xenobiotics nonetheless exists via these routes. The liver, however, as stated, can be involved in a not so insignificant recirculation process that is termed enterohepatic cycling. In this process xenobiotics that are secreted into the bile and excreted into the intestinal contents are reabsorbed efficiently and quickly from the chyme and redirected via the portal blood back to the liver for secretion again into the bile. Substances can persist in this cycle for a large number of iterations. The end result of this cyclic process is that the elimination half-life of a given xenobiotic can be significantly prolonged and toxicity to the gastrointestinal tract as well as other organs can be amplified.

GASTROINTESTINAL MOTILITY

As substances are permitted entry into the gastrointestinal tract, a variety of secretions are added and for their presence to be effective, mixing must occur [15,28,55, 58,67,70,74–76,83,99,102,129,132,133,139,144,155,162,165,170,180,181,183,190,193, 194,205,207,210,212,222,223,237,242,248,251,255–257,259,260,314,328,329,354,

355,361,363,365,378,383,384,390,395,405,411]. The proper and adequate degree of mixing of gastrointestinal contents facilitates both digestion and absorption. Materials must also be moved along the length of the GI tract to optimize absorption, take opportunity of different regional absorptive capabilities of the gastrointestinal tract, and eventually eliminate the waste stream. Indeed for these activities to take place, the gastrointestinal tract must be a very physically active organ. The gastrointestinal tract is in a continuous state of contractile activity, mixing ingesta via segmental contractions and then moving them along in an aboral fashion by a series of peristaltic contractions. The overall control of these activities is very complicated and not totally understood. However, it is known that contributions are made by the enteric nervous system, central nervous system, humoral agents, and the muscle itself. The central nervous system achieves its effects by means of autonomic nerves, somatic nerves, and humoral pathways.

As described previously, the gastrointestinal tract is invested with smooth muscle that extends both longitudinally down the tract and circularly enwraps the tract. The fibers within each bundle of muscle are interconnected via large numbers of gap junctions, which permit the facile transfer of electrical currents and communication due to the low-resistance movement of ions from one cell to the next. Accordingly, electrical signals that initiate muscular contraction can spread readily from one muscular fiber to another. While the smooth muscle fibers are separated from one another by loose connective tissue, they are still joined together at many points, forming a woven-like mass that functions as would be seen in a syncytium. So when an action potential is initiated anywhere within the muscle, it radiates outward in all directions, but more quickly along the long axis of the muscle fiber as opposed to the transverse axis of the muscle fiber. However, the distance that the action potential travels is dependent on the excitability of the muscle and if a muscle is not in an electronically excitable state then it will not respond to stimulation. Connections also exist between the circular and longitudinal muscle layers, so that different layers can excite each other as well.

The smooth muscle of the gastrointestinal tract has a slow, continuous level of electrical activity taking place along its membranes and there are two types of this activity, slow waves and spikes. The resting membrane potential of gastrointestinal smooth muscle is also not fixed and is capable of change itself, which can translate into changes of the motor activity of the gastrointestinal tract. Gastrointestinal contractions are rhythmic in nature and the character of this rhythm is determined essentially by the frequency and character of the slow waves. The cause of slow waves is not really known; however, this activity may be the result of a cyclical variation in the action of a sodium-potassium pump. Slow waves are not true action potentials causing muscle contraction except possibly in the stomach, but rather are slow persistent sinusoidal-like cycling changes of the resting membrane potential. The resting membrane potential can shift to become more hyperpolarized or depolarized in nature. The more depolarized the voltage about which the cycling takes place will dictate the frequency of appearance of spike potential waves, which in turn stimulate muscle contraction. The intensities of contractions do vary with region of the gastrointestinal tract.

Spike waves are action potentials and generally occur when the resting membrane potential of gastrointestinal smooth muscle becomes more positive than approximately -40 mV. Again, the more the slow wave potential rises above this level, the greater the frequency at which spike potentials occur. Spike potentials can occur at frequency of about 1 to 10 per second and in gastrointestinal muscle are of greater duration than action potentials observed in nerve fibers by a factor of 10–40. It is worth noting that the mode of generation of action potentials in nerve fibers is different than that in gastrointestinal smooth muscle. In nerves, action potentials are caused by the rapid flow of extracellular sodium into the fiber, whereas in gastrointestinal smooth muscle large amounts of calcium along with smaller amounts of sodium enter the cells. The gates through which this flux occurs are termed calcium-sodium channels, which open and close more slowly than the channels of nerve fibers. This slowness of action explains the long duration of action potentials in gastrointestinal smooth muscle. Notably the flux of calcium as expected plays a significant role in the muscle contraction itself. It was mentioned earlier that the resting membrane voltage can also change. Under general conditions, the normal resting membrane potential is about -56 mV. Factors that can make the membrane less negative than this baseline value or more excitable are stimulation by gastrointestinal hormones, parasympathetic nerve stimulation, physical stretching of the muscle and the presence of the neurotransmitter acetylcholine. The presence of epinephrine, norepinephrine, and stimulation of the sympathetic nerves can make the membrane more refractory or negative. It is of sufficient importance to bear repeating that the peaks of slow waves generate spike or action potentials and that the large quantities of calcium that concomitantly enter the muscle fibers cause most of the contractile activity.

The gastrointestinal tract has a self-contained nervous system, known as the enteric nervous system, which is located in the wall of the alimentary canal, starting in the esophagus and extending through the colon and controls gastrointestinal movements and secretion. This system is composed of two different plexuses, an outer or myenteric plexus and an inner or submucosal plexus. The myenteric plexus lies between the circular and longitudinal muscle layers and the submucosal plexus resides in the submucosa. Gastrointestinal movements such as the intensity of contraction, increased tonicity of contraction, rate of contractile rhythm, and velocity of conduction of excitatory waves along the gastrointestinal tract wall are controlled mostly by the myenteric plexus. The myenteric plexus also releases through fiber endings vasoactive intestinal polypeptide, which inhibits intestinal sphincter muscle activity and accordingly slows the movement of gastrointestinal contents along the tract and the emptying of contents from the stomach into the intestine as well as the emptying of contents from the small intestine into the large intestine. Blood flow, secretion, local absorption, and local contraction that modulate the degree of in-folding of the gastrointestinal mucosa are controlled by the submucosal plexus. Some degree of intercommunication exists between the two systems and sympathetic and parasympathetic fibers connect with both plexuses. While the enteric nervous system can and does function independently, signals from the parasympathetic or sympathetic systems can activate or inhibit gastrointestinal functions. Stimulation of the parasympathetic nerves causes a general increase in activity of

the complete enteric nervous system, enhancing the activity of most gastrointestinal functions. Stimulation of the sympathetic nerves inhibits activity of the gastrointestinal tract, generally causing a decrease in the activity of most gastrointestinal functions. Sensory nerve fibers originating in the gastrointestinal mucosal epithelium send fibers to both enteric plexuses, the vagus nerves, the parvertebral ganglia, and spinal cord.

The stomach has three motor functions. The first of these is storage of food until it can be processed in the lower gastrointestinal tract. The next is to mix food with gastric secretions turning it into chyme. The third and last is to slowly empty the chyme into the small intestine at a rate which permits adequate digestion and absorption by the small bowel. If the stomach is empty intense contractions can occur and these contractions generally start 12–24 hours after the last ingestion of food. These contractions are aborally moving rhythmic peristaltic contractions occurring in the body of the stomach that can sustain themselves in tetany for periods of time of up to 3 minutes. These contractions can be influenced by low levels of blood sugar. When the stomach contains food, constrictor or mixing waves originate in the middle portion of the stomach wall and travel toward the antrum of the stomach at a frequency of about one every 15–20 seconds. As these waves move from the body to the antrum, they become ring-like constrictions of the gastric wall and of increasingly greater intensity that force under high pressure the gastric contents toward the pylorus. These contractions can create forces of 50–70 cm of water pressure and each time a peristaltic wave moves toward the antrum, it presses firmly against the antral contents. However, the opening of the pylorus is sufficiently small enough in size that only a few milliliters of chyme are introduced into the duodenum with each compressing peristaltic wave. In its normal resting state, the pyloric sphincter usually remains sufficiently open for liquids to empty from the stomach without resistance. However, this formidable barrier prevents the passage of food into the small bowel until it has been adequately mixed. Keep in mind that as each peristaltic wave approaches the pylorus, the pyloric muscle itself contracts. This activity inhibits the expulsion of gastric contents into the duodenum and accordingly antral contents are ejected upstream through a peristaltic ring back toward the body of the stomach. This process is repeated over and over, permitting a great degree of mixing in the stomach and reduction of the food mass to a fluid-like consistency. As the stomach slowly begins to empty, contractions initiate further up the body of the stomach wall, gradually isolating the food into the lower portions of the stomach. This mixing and propelling activity is sometimes referred to as the pyloric pump.

The rate of emptying of the stomach is controlled via signals from the stomach and duodenum, with the latter providing the more significant level of control, which assures adequate digestion and absorption in the small intestine. The gastric factors that stimulate emptying of the stomach are the volume of gastric contents and the presence of the hormone gastrin. Duodenal-based factors which inhibit gastric emptying are osmolarity of the chyme, degree of distension of the duodenum itself, acidity of the chyme, irritation of duodenal mucosa, degradation products of proteins, the degradation products of fats and hormones released from the small intestine (cholecystokinin [CCK], gastric inhibitory peptide [GIP], and secretin).

Similar to the stomach, the small intestine possesses muscular contracting activities which facilitate both the mixing and propulsion of its contents. Segmental contractions ensure proper mixing, whereas peristaltic contractions move the chyme along the tract in an aboral direction. It is important to realize though that the mixing activity is never pure mixing and similarly as regards propulsion, propulsion is never purely propulsion and there is a great deal of overlap between the two. Reduced segmental activity can speed transit times, while reduced peristaltic activity can prolong transit times. As the wall of the small bowel becomes distended, this stretching stimulates the initiation of local ring-like contractions at various locations and intervals along the small intestine. Each of these contractions resembles or acts much like a hand squeezing a sausage-shaped balloon. These contractions can be isolated, irregularly spaced, regularly spaced or weak and irregularly spaced and occur at a frequency of about 12 per minute in the duodenum and jejunum and at a frequency of about 9 per minute in the ileum. The slow waves in the smooth muscle of the intestinal wall play an important role in the maintenance of segmental contracting activity, but the assistance of and contribution from the enteric nervous system is essential for normal function.

Peristaltic waves can occur in any part of the small intestine, producing a net aboral movement of contents at an average velocity of about 1 cm per second (0.5–2.0 cm/sec), but movement is somewhat faster in the more proximal segment of the small intestine than in the terminal portion. This means that about 2–5 hours are required for chyme to enter the duodenum and reach the ileocolic sphincter. These waves too are initiated by distension of the intestinal wall and typically travel only about 3–5 cm and only rarely further than 10 cm. They are characterized by the formation of ring-like contractions, which move aborally pushing the intestinal contents along the long axis of the gastrointestinal tract. These contractions much resemble the action of a pair of pinched fingers working to extrude toothpaste from a tube. With the proper type of stimulation (toxins, irritation, etc.) peristaltic and segmental contractions can significantly increase in magnitude and frequency. Peristalsis not only moves contents along the small intestine toward eventual elimination but also spreads out the chyme along the intestinal mucosa facilitating the processes of digestion and absorption. Peristalsis is significantly increased after a meal and subject to both neural and hormonal control. The stimuli initiating peristalsis are the entry of chyme into the duodenum and more significantly the gastroenteric reflex. The gastroenteric reflex is started with distension of the stomach and impulses sent from the stomach through the myenteric plexus to and along the walls of the small intestine. Gastrin, serotonin, insulin, and cholecystokinin enhance intestinal motility. Secretin and glucagon inhibit intestinal motility. Other neurotransmitters have been implicated in gastrointestinal function, but little is known about their roles and functions. These substances would include somatostatin, bombesin, dopamine, adenosine triphosphate, leu-enkephalin, and met-enkephalin.

The muscularis mucosa mentioned in the section on anatomy can cause the appearance of folds of varying length to appear in the intestinal mucosa. The appearance of folds increases the absorptive surface area and hence the rate of absorption. These same muscularis mucosa fibers also extend into the intestinal villi, causing

them to extend or contract, again increasing or decreasing the available absorptive surface of the small intestine. Local nervous reflexes mediate the mucosal and villous contractions. The repeated cycle of villus elongation and contraction, aids in the drainage of lymph from the central lacteal of the villus to the lymphatic system.

As chyme moves along the small intestine, it may eventually become blocked at the ileocolic sphincter. However, the consumption of an additional meal can stimulate the gastroileal reflex and propulsion of the chyme through the ileocolic sphincter valve into the large intestine, which receives up to approximately 1500 mL of chyme each day. The ileocolic sphincter has two main functions. The first is to prevent the backwash of colonic contents into the small intestine and the second is to prolong the residence time of intestinal contents so as to enhance absorption. The ileocolic sphincter is of sufficient strength so as to withstand anterograde pressures of approximately 50 cm of water. The proximal half of the entire colon is designed for absorption and the distal half primarily for storage. Accordingly, the movements of the colon are very slow in nature, but despite this sluggish nature, the same movements as exhibited in the small intestine are present in the large intestine. The mixing contractions can almost momentarily obliterate the colonic lumen and the combined action of the circular and longitudinal muscles work to persistently turn over the fecal contents, much like the tilling of a garden, constantly exposing new material to the colonic absorptive surface. Propulsive contractions progressively move the increasingly more solid fecal stream toward the rectum over a time period of 8–15 hours. Typically, about 80–200 mL of feces are expelled each day.

Mass movement is a modified type of peristalsis in which distal colonic contents are forced into the rectum. As the colon becomes distended, constrictive rings form and travel in such a fashion as with the small intestine to move colonic contents further along the colon. These constrictive rings propagate for a distance of about 20 cm with the propulsive activity lasting for approximately 30 seconds, followed by 2–3 minutes of relaxation until the cycle begins anew. Mass movements typically last for about 30 minutes and do not recur until about 12–24 hours later. Mass movements are stimulated by the gastrocolic and duodenocolic reflexes, which in turn are initiated by distension of the stomach and duodenum, respectively. Transit times for the dog have been reported by Davies and Morris to be about 96 minutes through the stomach, approximately 110 minutes through the small intestine and about 770 minutes through the entire length of gut.

Reference has been made in the previous text to different gastrointestinal reflexes, such as the enterogastric, gastrocolic, duodenocolic, and gastroileal reflexes, which can affect motility of the gastrointestinal tract. There are basically three different types of gastrointestinal reflexes. The first group of these are reflexes of the enteric nervous system, which are contained within the enteric nervous system and involve such activities as contraction, peristalsis, local effects, mixing and secretion. The second class of these are reflexes are those that travel from the wall of the gastrointestinal system to the paravertebral sympathetic ganglia and then back to the wall of the gut. These reflexes send signals over long distances and are involved in such functions as signals from the stomach to elicit evacuation of the colon. The third and last group of these reflexes are cycles which involve the travel of impulses from the

wall of the gastrointestinal tract to the spinal cord or brain stem and then back to the gut wall. These reflexes can initiate gastric motor or secretory activity via the vagus nerves, defecation or involve the sensing of pain and the development of a response to it. Pain reflexes typically invoke a general inhibition of gastrointestinal tract activity. There are a number of reflexes which have not been referenced in the text such as the peritoneointestinal, vesicointestinal, renointestinal, and somatointestinal, which can all inhibit gastrointestinal motility.

BACTERIAL FLORA

Any discussion of the gastrointestinal tract would not be complete without some attention being paid to bacterial flora [175,227,238,250,254,315,320,321,347,385]. The digestion and absorption of carbohydrates, fats, proteins, peptides, amino acids, and vitamins are all highly influenced by the presence of a healthy microbial population. Indeed, some short-chain fatty acids produced by bacteria can even stimulate gastrointestinal secretory activity. The resident microbial population works to prevent the colonization of pathogens and provides a low level of stimulation for the enteric immune system. Indeed, the presence of a normal, functioning and healthy population of intestinal bacteria can affect such intestinal characteristics as intestinal motility, rate of enterocyte turnover, rate of microvillous enzyme turnover, and even the size of villi. The total population of microbial flora increases as one travels aborally from the duodenum to the colon. The maintenance of this gradient of microbial numbers is the result of many factors, including patency of the ileo-ceco-colic valves, degree of intestinal motility, amounts of bacteriostatic or bacteriocidal secretions (e.g., gastric acid, pancreatic secretions, bile), substrate type, substrate availability, and intestinal lumen size and patency. An imbalance of any of these factors or extreme divergence from the normal homeostatic state can cause the overgrowth of small intestinal bacteria, an undesirable situation with adverse consequences. Indeed the loss of tolerance of the normal population of flora can initiate inflammation of the intestine, dysfunction of the intestine and even neoplasia. The normal canine small intestinal microbial population includes, but is not limited to aerobes, anaerobes, and facultative anaerobes. *Proteus* spp., *Escherichia coli*, *Bacillus* spp., *Staphylococcus* spp., *Corynebacterium* spp., *Enterococcus* spp., *Enterobacteriaceae*, and *Streptococcus* spp. are common aerobes. *Clostridium* and *Bacteroides* spp. are common anaerobes. Healthy dogs have approximately 10^9 colony-forming units of aerobic and anaerobic bacteria per milliliter of undiluted gastrointestinal juice in the proximal portion of the small intestine. The presence of xenobiotics, especially antibiotics, can affect not only the total count of bacteria in the small intestine but also the relative populations of the microbes. However, resident bacterial populations in the small intestine do appear to be relatively resistant to changes in the diet. While qualitative and quantitative differences do exist, microflora in animals is strikingly similar, but exceptions to this general rule have been noted. First, the number of bacteria in the canine intestinal tract exceed by several orders of magnitude that found in humans, so caution should be used when comparatively discussing

matters impacting bacterial flora in humans and dogs. As microbes can be expected to contribute to the breakdown, metabolism, and modification of xenobiotics, species differences in bacterial populations can be important. An example might be the presence of bacterial beta-glucosidase activity in the proximal portion of the small intestine. If this activity is nonexistent or significantly decreased due to low numbers of relevant microflora, the aglycone portion of a xenobiotic may not be absorbed or may be absorbed at a significantly reduced level.

The large intestine, however, contains the greatest amount of bacteria of any region in the canine gastrointestinal tract. Typically 10^{11} organisms can be found per gram of feces. *Streptococci* spp. and *Enterobacteria* spp. are the predominant aerobes found in the large intestine and *Lactobacilli* spp., *Clostridia* spp., *Bacteroides* spp., *bifidobacteria* are the predominant anaerobes. However, over 90% of the microbial population in the large intestine is anaerobic in nature. As in the small intestine, a variety of factors are necessary to remain within proper balance to prevent bacterial overgrowth and the development of ensuing pathology. These factors would include normal intestinal motility, existence of a normal coating of mucus on the mucosa, presence of adequate dietary nutrients, levels of oxygen, and the presence of bacterial interactions suitable to the fostering of a normal stable microbial population. The presence of xenobiotics, especially antibiotics, can upset this balance as do also increased levels of bile salts. The microflora present in the large intestine serves an important function in the digestion of remaining undigested proteins and carbohydrates. Luminal bacteria degrade protein and carbohydrate nutrients to short-chain fatty acids like butyrate, acetate, and propionate, which are quickly absorbed by the intestinal mucosa. The acetate and propionate are utilized by hepatocytes for the synthesis of triglycerides and cholesterol as well as for the production of energy. Butyric acid is the preferred energy source for colonic epithelial cells and is essential for their normal growth and function. Collectively, the short-chain fatty acids contribute to the maintenance of the acidic luminal pH. Such acidification prevents the formation of potential large intestinal corrosive agents and irritants such as ammonia, ionized bile acids, and ionized long-chain fatty acids, which if present in significant amounts can lead to dysfunction of the large intestine and the development of pathology.

We have mentioned the presence of xenobiotics, their potential for a direct killing effect on microbes in the gastrointestinal tract and the development of ensuing pathology. This killing can be selective, favoring a particular species of bacterium or group or alternatively can be broad spectrum in nature thereby eradicating the bacterial population as a whole. In the former case bacterial overgrowth can occur, which can lead to a loss of the homeostasis that exists in the gut. At the other extreme is the total obliteration of bacteria in the gut. As discussed, neither of these options is desirable and both can lead to the development of overgrowth of pathogenic organisms. However, there are other toxicology-related consequences that should be considered with regard to the presence of xenobiotics and their potential interaction with gastrointestinal microflora. Bacteria can function in such a fashion as to produce toxic metabolites xenobiotics and even active carcinogens. These metabolites can be very different from those found in tissues and the system of an organism as a whole

and their concentrations can be highly variable in amount. Small, even insignificant levels can lead to the development of notable toxicities. Alternatively, xenobiotics can be detoxified by microbes in the gastrointestinal tract or even converted into other pharmacologically active metabolites, with similar or different types of activity. The microbial metabolism of xenobiotics can lead to the production of metabolites, which are able to undergo enterohepatic circulation, thereby resulting in the increased exposure to the breakdown products of the parent. Finally, as a result of the differences in the genetics and metabolic activities of varying components of the total population of gastrointestinal bacteria, different toxicity profiles might emerge. This could be noted as differences in the character or degree of toxicity between species or individuals within a species. An example is the reduction of aromatic nitro groups to aromatic amines by bacterial flora. These compounds can be goitrogenic or carcinogenic in nature.

PATHOPHYSIOLOGY AND TOXICOLOGY OF THE GASTROINTESTINAL TRACT

It has been stated previously, but bears repeating that the gastrointestinal tract communicates with the external environment and as such is exposed to a wide variety of substances. Indeed, when it comes to pure exposure, the gastrointestinal tract is at the very least not surpassed by any other organ of the body in terms of the broad spectrum of materials to which it can be exposed. As the gastrointestinal tract is a dynamic organ playing a key role in the absorption of nutrients and facilitating the passage of ingesta, digesta, chyme, and feces through the body, the gastrointestinal tract finds itself very susceptible to injury via the same processes that permit it to provide nutrition to the body. There are a variety of mechanisms by which damage to the gastrointestinal tract can occur and these include immune reactions, enterocyte necrosis, accelerated apoptosis of epithelial cells, destruction or impairment of function of mucosal stem cells, stimulation of division of mucosal stem cells, direct cytotoxicity, genetic toxicity, corrosion, irritation, reduction in the supply of oxygen (ischemia or hypoxia), reduction in blood supply, generation of reactive oxygen moieties, hypersensitivity, damage to enteric nervous system, compromise of the brush border of enterocytes, enzyme inhibition, enzyme activation, disruption of intracellular signaling processes, altered permeability of the mucosal layer, destruction of the mucus barrier, increased susceptibility to acid, inhibition of production of important humoral agents (e.g., prostaglandins), stimulation of production of important humoral agents (e.g., gastrin), and alteration of transport mechanisms. There can also be delayed consequences of toxic exposure such as the development of neoplasia [5,7,11,14,20,78,92,130,154,156,160,178,187,227,249,258,293,295,298,303,313,318,327,330,339,343,353,356,358,392,400,407,414,416,417].

While some agents can cause toxicity of the gastrointestinal tract by means of a single mechanism, most do not and rather involve multiple mechanisms. These mechanisms can be concurrent or sequential and can move toward a single endpoint or different end points. Furthermore, due to regional differences in structure and

function within the gastrointestinal tract, manifestations of toxicity can vary with the geographic location of exposure. That said, it is fair to state the toxic responses of the gastrointestinal tract can generally be classified as being inflammatory in nature, erosive in nature or stimulatory in nature. Obviously the gross characterization of any toxic exposure is dependent upon a number of factors such as the duration of exposure, the size of the dose, the extent of gastrointestinal damage, the types of cells or tissues involved in the damage, the mechanism of action of the offending agent, and the accommodation of injury or the ability of the gastrointestinal tract to implement repair in a timely fashion. At a microscopic level, the toxicity profile is dictated at least in part by the ability of individual cells to retain viability, maintain internal homeostasis, generate energy, reseal damaged membranes and replace lost, damaged, or nonfunctional components.

Most materials, regardless of toxicity, that are consumed end up being absorbed into the body by either passing directly through enterocytes or around them via passive paracellular diffusion. This passage itself however is not without challenge as an agent must move from the aqueous milieu of the lumen through the nonstirred water layer, mucus layer, and epithelial cells to gain access to the circulation. The physico-chemical characteristics of any substance are very important features in the successful navigation of this movement, with large polar molecules poorly penetrating uncompromised normal epithelium with functional tight junctions. Tight junctions are of varying permeability, depending upon their location within the intestine, and become progressively more impermeable the more aborally one travels. Weak acids and weak bases as previously discussed can exist in an equilibrium which permits or does not favor absorption. Electrically neutral and small molecules can quickly move around epithelial cells to accomplish absorption. While specific transporters can play an important role in absorption, if no such system exists for a substance, it can be passively absorbed between epithelial cells, through the tight junctions joining the cells together. Moieties that gain access inside of an enterocyte can be extruded via various transporter systems back into the intestinal lumen, permitted full absorption, metabolized or eliminated. Metabolism, regardless of the site can result in the manufacture of substances that are equally, more, or less toxic than the parent compound. This brief review of the penetration of the gastrointestinal mucosal epithelial barrier function and associated absorptive activities serves to emphasize the complexity of gastrointestinal epithelial mucosal function and interaction. Subsequently, it would appear that the profiles of toxicity of the gastrointestinal tract might be complex also. However, the clinical signs of gastrointestinal toxicity, distilled to the essence are vomiting, diarrhea (with or without blood) or/and malabsorption. Strangely enough, while vomiting and diarrhea are considered primary responses to the presence of toxic agents, vomiting and diarrhea can also be considered to be the first lines of defense in protection against toxic insult.

The act of emesis or vomiting can be a toxic response, but can also generally be thought of as being a reflex, to protect the stomach and intestines from the intromission of toxic substances and prevent deeper penetration into the alimentary canal and a greater degree of absorption [8,212,213,233,288,357,359,360]. Typically associated with emesis is nausea, which is hard to assess in the canine. Typical signs of

nausea in the canine can include excess salivation, dullness, lack of responsiveness, depression, a lack of interest in food and vomiting. The dog is considered to be very susceptible to vomition and hence a somewhat detailed discussion of this activity is in order. The emetic process consists of three phases, pre-ejection, retching, and ejection. In the pre-ejection portion, gastric relaxation and retrograde peristalsis occur. Retching involves the rhythmic action of respiratory muscles preparatory to actual act of vomiting and involves the contraction of intercostal, abdominal and diaphragmatic muscles, and relaxation of the upper esophageal sphincter. Accompanying this activity can be shivering and salivation, driven by the autonomic system. If this activity is prolonged, behavioral changes can occur along with the dullness and depression. All of this activity is thought to be coordinated by a central emetic center residing in the lateral reticular formation of the middle portion of the brainstem adjacent to the chemo-receptor trigger zone in the area postrema, beneath the floor of the fourth ventricle and the vagal nucleus tractus solitarius. It is important to mention that there is no blood-brain barrier around the chemo-receptor trigger zone (CTZ) and this permits a persistent analysis of substances in the blood or cerebro-spinal fluid by the CTZ. Upon proper stimulation, the CTZ can send signals to the emetic center to initiate the protective process of emesis. Splanchnic afferents and the vagus nerve also provide input to the emetic center along with the cerebral cortex and the vestibular apparatus. The vestibular apparatus is generally associated with motion-related stimulation. The contribution of the cerebral cortex is not well-defined in the canine, but is felt to nonetheless be involved. The emetic center communicates via appropriate efferents to generate relevant smooth muscle, skeletal muscle, vasomotor, respiratory, and salivary activities, which are all part of the process of emesis. Specific efferent information sent to the gastrointestinal tract results in the stimulation of retrograde duodenal contractions, retrograde gastric contractions, relaxation of the caudal esophageal sphincter, gastroesophageal reflux, opening of the proximal esophageal sphincter and the evacuation of gastrointestinal contents.

High concentrations of norepinephrine, serotonin (5-hydroxytryptamine), acetylcholine, opioid, histamine, substance P, neurokinins, enkephalins, and dopamine receptors exist in the chemo-receptor trigger zone. Specific receptor subpopulation types within these groups are α_2 adrenergic, 5-hydroxytryptamine₃ (5-HT₃) serotonergic, M₁ cholinergic, H₁ and H₂ histaminergic, neurokinin 1 (NK₁) neurokininergic, enkephalin mu (ENK _{μ}) and enkephalin delta (ENK _{δ}) enkephalinergic, and D₂ dopaminergic. Associated with the presence of these neurotransmitters are various synthetic or degradative enzymes, including, 5-hydroxytryptophan decarboxylase, choline acetyltransferase, histidine decarboxylase, aminopeptidase, enkephalinase, dopa decarboxylase and dopamine-hydroxylase. Considering the presence of all of these receptors and agonists/antagonists the emetic process is apparently a very complex one. There is undoubtedly some sort of hierarchy between the myriad neurotransmitter and receptor pathways, but the relative levels of priority the mechanisms of action are not unequivocally defined at this time. The emetic center itself has been found to contain primarily α_2 -adrenergic receptors (α_2) and 5-hydroxytryptamine_{1A} (5-HT_{1A}) as the major receptors involved in the control of emesis exerted by the

emetic center. The vestibular apparatus more than likely has a mixed population of histamine (H_1) and muscarinic (M_1) cholinergic receptors. Opioid (ENK_{μ}) and benzodiazepine (ω_2) receptors are purportedly present in the cerebral cortex, but their contributions are not well-recognized and may even play only minor roles in the emetic process. The nucleus, tractus solitarius is populated with many histamine, neurokinin (NK_1) and cholinergic receptors along with some 5-HT₃ receptors.

In the gastrointestinal tract itself, specific toxins, corrosive activity, general irritation, mechanical irritation, inflammation, luminal distension, cell necrosis, accelerated apoptosis, or any loss of cells can cause emesis. Accordingly, many different types of receptors are found in the gastrointestinal tract, such as motilin (MOT), muscarinic₂ cholinergic (M_2), 5-hydroxytryptamine₄ (5-HT₄), neurokinin₁, and 5-hydroxytryptamine₃ (5-HT₃). Within the gastrointestinal tract, the 5-hydroxytryptamine₃ (5-HT₃) receptors more than likely play the most important role in the initiation of emesis. Drugs that are cytotoxic by nature bring about the release of 5-hydroxytryptamine from enterochromaffin cells in the gastrointestinal tract. The 5-hydroxytryptamine in turn then activates 5-HT₃ receptors in vagal afferents, which in turn stimulate the nucleus tractus solitarius, which then stimulates the emetic center. It has not been unequivocally established whether or not the release of 5-hydroxytryptamine is associated with inflammation, irritation, corrosion, the presence of specific toxins, cell necrosis, cell loss, and luminal distension.

The variety of motor activities performed by various parts of the gastrointestinal tract that cause vomiting are mediated by signals from vagal efferents and myenteric neurons. These signals cause either excitation or inhibition of smooth muscle. Emptying of the stomach and intestinal transit are at least in part controlled by receptors located on myenteric neurons and gastrointestinal smooth muscle. These receptors include those that are neuronal-based, D₂-dopaminergic and 5-hydroxytryptamine₄ as well as those that are smooth muscle-based, motilin and M₂ muscarinic cholinergic.

It is important to realize that there are really only two ways of inducing emesis, one is via a humoral pathway and the other is via a neural pathway. In the humoral pathway, the chemo-receptor trigger zone is stimulated by the presence of substances in the blood, which are emetogenic. In the neural pathway, the emetic center can be stimulated by (1) vestibular input (motion) to the cerebellum; (2) sensory (pain, smell, sight) input to the cerebral cortex; (3) memories (fear) in the cerebral cortex; (4) mechanical stimulation such as pharyngeal gagging, which results in the sending of signals through glossopharyngeal and trigeminal afferents to the nucleus tractus solitarius; (5) vagal and sympathetic afferents from the gastrointestinal tract (stomach and small intestine) which carry signals as a result of direct stimulation or irritation to the nucleus tractus solitarius. With the inputs identified above, the cerebral cortex, cerebellum and nucleus tractus solitarius can initiate activity in the emetic center, which can result in the process of vomiting. Vestibular and sensory inputs as well as memory undoubtedly provide only a small contribution to the emetic process for the canine. Antagonism of the chemo-receptor trigger zone can abolish emetic activity stimulated by the presence in blood of emetogenic substances, but emetic center antagonism, vagotomy, and sympathectomy are not helpful in abolition

of emetic activity brought about by the presence of blood-borne emetogues. Alternatively, gastrointestinal pathology or toxicity-induced emetic activity can be abolished via emetic center antagonism, sympathectomy or vagotomy, but not by antagonism of the chemo-receptor trigger zone. This redundancy of pathways underscores the importance of this process as a method of protection. However, a complete, well-defined uniformly accepted process for emesis has still not been defined.

Emesis may occur as a result of or in association with not only toxicity but also with a number of different medical conditions, such as infection or infectious conditions, inflammation, neoplasia, uremia, hepatic failure, hypercalcemia, septicemia, endotoxemia, systemic organ failure, hyperthyroidism, and hypoadrenocorticism. Chemical substances that can be associated with emesis include opioids, aminoglycosides, cytotoxic drugs, cholinergic mimetics, L-DOPA, bromocriptine, macrolide antibiotics, and digitalis glycosides. Radiation can also induce emesis.

Although a toxic response, diarrhea can also be considered to be a protective mechanism that occurs if a material or materials that are potentially toxic evade emesis and penetrate more deeply into the gastrointestinal tract than the stomach [59,109,120,141,221]. The pathophysiology of diarrhea can have a variety of different origins or etiologies, with the onset and progression being the result of many events occurring at the same time or consecutive events occurring in a sequential and dependent fashion. For example, pancreatic toxicity leading can lead to exocrine pancreatic insufficiency and the resulting intestinal bacterial overgrowth can lead to inadequate digestion of intestinal contents, hyperosmolarity, and irritation. Another example might be the administration of an antibiotic, which can cause bacterial overgrowth or a shift in the relative proportions of populations of flora. If the enteropathogenic bacteria *Escherichia coli* take opportunistic advantage of this situation, they will produce in toxic amounts a heat-stable enterotoxin, which will stimulate in enterocytes the production of cyclic guanosine monophosphate (cGMP) via the activity of guanylate cyclase. The presence of cGMP in turn activates guanosine monophosphate-dependent protein kinases, which result in the production of a profuse secretory diarrhea. Consequently and concurrently, platelet-activating factor, prostaglandins, and leukotrienes are locally released, which can contribute to the development of disturbances of permeability, intestinal motility, and malabsorption. In any case of bacterial overgrowth, the potential exists for higher numbers of different types of microbes to bring about the bacterial degradation of enzymes admixed into the intestinal stream. If fat digestion and absorption are primarily affected, then a fatty-type of diarrhea or steatorrhea develops. Diarrheas of small intestinal origin are characterized by hypersecretion, altered mucosal permeability, and dysfunctional absorption or malabsorption, but diarrheas of large intestinal origin are characterized by large intestinal mucosal injury, large intestinal altered or malabsorption and hypersecretion.

If a substance is not completely or sufficiently expelled via emetic activity or diarrhea from the body and establishes a presence in the gastrointestinal tract, there are a variety of responses in addition to vomiting and diarrhea which can become manifest by the gastrointestinal tract. In some cases, these are generalized responses typical of the entire length of the gastrointestinal tract, while in others the responses

may be regionally specific. These responses generally can occur in conjunction with or exclusive to vomiting or diarrhea. However, vomiting and diarrhea not uncommonly develop as each pathogenic mechanism pursues its course. Brief discussions of these responses follow [13,39,44,49,53,57,60,88,89,106,121,145,151,152,161,166,177,191,199,201,216,234,239,243,247,264,271,275,276,289,292,320,326,352,367,388,397,404,408].

As stated previously, the mucosa of the gastrointestinal tract comprises a protective barrier to external insult, while at the same time performing functions vital to complete digestion and efficient absorption. The gastrointestinal tract has as part of its mucosal population extensive areas where there are rapidly dividing stem cells. This is strange, because of the hostile environment in which these cells reside. Other rapidly dividing stem cells in the body such as hematopoietic cells of the marrow and sperm precursors in the testes exist in highly protected environments. However, despite this lack of protection, these stem cells and the mucosa of the gastrointestinal tract are not as frequent a site of toxic injury as one might expect. Some possible explanations for this finding are the fact that intestinal contents are dilute, a protective mucus layer covers the entire mucosa, epithelial cell membranes are enriched with membrane strengthening glycosphingolipids, the mucosal cell lifetimes are relatively short and the large total surface area permits frequent and intimate contact of xenobiotics with cells capable of bringing about detoxifying biotransformation. The effectors of biotransformation can be membrane-bound or cytosolic in location and of a variety of different types. Despite these protective mechanisms, if irritation of the mucosa does occur, increased levels of prostaglandins can be produced, which protect the mucosal cells through preserving blood flow and stimulating the secretion of bicarbonate into the small intestine. If mucosal cells are not terminally damaged, they can retain viability through a process of resealing their damaged membranes. In general, mucosal cells are very effective in modulating the basal rate of mucosal cell proliferation so as to maintain a fully competent mucosal epithelial barrier. Indeed, in the presence of toxic substances, the lifetime of an enterocyte is reduced and with the passage of the toxic threat, the parameters of epithelial mucosal replacement return to normal within 72 hours.

Toxic substances can cause the death or loss of enterocytes. Regardless of the cause, loss of cells from the epithelial population can impede the overall ability of the gastrointestinal tract to transport chloride ion, conduct sodium/glucose cotransportation or any other aspects of absorption or secretion that are dependent on cellular signal transduction pathways. These activities are mediated by opioid, norepinephrine, 5-hydroxytryptamine, acetylcholine, and prostaglandin E₂ receptors located on enterocyte membranes.

Disruption of the intestinal mucosal barrier can result in a loss of tolerance and resistance of the intestinal epithelium to the presence of acids, bacteria, and enzymes, which can result in the development of inflammation, corrosion, ulcer formation, ischemia, and cytotoxicity. In cases of dysfunction and malabsorption in the small intestine, substances that are susceptible to fermentation are moved along to the large intestine, where they are converted to products that exhibit a greater degree of osmotic activity. This activity leads to the damage of large intestinal

mucosa with subsequent inflammation. Mucosal damage results in the development of altered mucosal permeability and the exudation of fluids into the intestinal lumen. Inflammation leads to the release of prostaglandins, which in turn stimulates the release of histamine and the secretion of electrolytes. The consequences of inflammation can cause altered absorption or malabsorption and the development of altered gastrointestinal motility.

Damage to the brush border of enterocytes can occur as a result of direct toxicity, inflammation, bacterial overgrowth or enzyme inhibition. Anaerobic bacteria are extremely proficient in causing destruction of the brush border and its associated enzymes and transport proteins. Brush border damage can result in maldigestion, dysfunctional absorption, and the development of possible bacterial overgrowth with complications of fermentation and the development of osmotic diarrhea.

Intestinal hypersensitivity can evoke the onset of diarrhea via the initiation of inflammation and the production of a wide variety of different chemical mediators. However, this topic is more completely discussed elsewhere in the section addressing immunology of the gastrointestinal tract [279].

Inflammation or the inflammatory response can occur as the sole component or complication of a toxic response of the gastrointestinal tract and has been implicated in the development of diarrhea and malabsorption. The development of inflammation should be no surprise, considering the hostile environment that exists throughout the total length of the lumen of the gastrointestinal tract. There are damaging enzymes, acid and millions of bacteria, which can speed the development of serious lesions, which are most commonly seen as ulcers. Intestinal inflammation can be initiated by any of a number of factors including bacterial overgrowth, epithelial irritation or abrasion, cell necrosis, cell loss, or the presence of specific directly toxic agents. While inflammatory responses associated with lesions of a pure toxicologic origin tend to not be severe, the complication of insult due to the persistent presence of acid, enzymes, and bacteria can radically change that pattern. Inflammation of the stomach is generally referred to as gastritis and that of the large intestine as colitis. Small intestinal inflammation is generally referred to as enteritis, but if regional specificity is found, the terms duodenitis, jejunitis or ileitis can be used. Stated simply, the presence and activities of various formed elements of the blood combined with appropriate levels of a whole host of chemical mediators work in a synergistic fashion to create an inflammatory response in the epithelial mucosa and submucosa. The inflammatory response incurs the presence of cellular infiltrates, accumulation of fluid (localized edema) and the alteration of epithelial barrier efficiency, intestinal motility, and mucosal permeability. Inflammation in the gastrointestinal tract is characterized by alterations in vascular diameter that lead to altered blood flow to the affected area, structural changes in the microvasculature that permit plasma proteins to leave the circulation (exudates or transudates), the emigration of leukocytes from the microcirculation, the accumulation of leukocytes at the focus of injury or insult, the activation of leukocytes, the secretion of large amounts of mucus, the development of ulcers, bleeding, and lymphoid hyperplasia. It should be obvious that pain and loss of function are involved with this process. Loss of function can include the impairment of many different mechanistic processes and the severity of loss of

function can be directly proportional to the severity of inflammation and the total amount of tissue damage sustained. Inflammation can be either acute or chronic in nature and can be characterized as such by the populations of cells accumulating in the area of interest. Cases of chronic inflammation usually have large numbers of lymphocytes, macrophages, and plasma cells present in the affected area. Levels of arachidonic acid metabolites from the cyclooxygenase and lipoxygenase pathways become elevated during inflammation. Thromboxane A₂ formed by platelets is one of these compounds and is a potent vasoconstrictor that can bring about extensive mucosal damage as a result of induced hypoxia, especially in the presence of the bile acid taurocholate. Vascular thrombosis and local infarcts can also result from the presence of increased levels of thromboxane A₂, which causes aggregations of platelets. Leukotrienes are additional products of the arachidonic cascade and also promote vasoconstriction and the decreased supply of oxygen. In the presence of a sufficient level of hypoxia or even ischemia, cells can become sloughed off the basement membrane and the formation of ulcers initiated. However, prostaglandins, prostacyclins, and lipoxins increase mucosal blood flow and improve the supply of oxygen, counteracting the oxygen deprivation actions of other compounds. It is easy to see that this is a process with a delicate balance that can be readily tipped to the disadvantage of the integrity of the gastrointestinal tract.

Regardless of the cause, the process of inflammation can involve the production of reactive oxygen species. While these free radicals can provide defensive protective effects, they can also create toxicity in the gastrointestinal tract. Neutrophils and macrophages produce hypochlorous acid and hydroxyl, superoxide, and peroxy radicals. Neutrophils, thrombocytes, and vascular endothelial cells produce nitric oxide. The generation of reactive oxygen species is an ongoing process in all tissues, but moieties are generated at very low levels and in the presence of protective measures. If the levels of reactive oxygen species rise above that for which adequate control can be provided, tissue damage naturally occurs. The presence of xenobiotics and the processes of inflammation can overwhelm the intracellular enzymes (catalase, superoxide dismutase, and glutathione(SH) peroxidase) and extracellular fluid-based antioxidants (vitamin A, vitamin E, selenium, β -carotenes, ascorbic acid, and α -tocopherols) normally present to prevent or limit the degree of oxidative damage to tissues and cells. Sequella to the presence of reactive oxygen species can include, altered blood flow, alterations of vascular permeability, direct cell toxicity, cell death, fluid secretion into the intestinal lumen, and cell loss.

When the accelerated loss of enterocytes from villi or the reduced supply of enterocytes from stem cells located in the bottoms of intestinal crypts occurs, atrophy of the villi and hence total available absorptive surface area occurs. Toxicity can target mature cells, maturing cells, or stem cells located deep in the intestinal crypts. While cells can make accommodations to preserve the intestinal barrier and absorptive functions, such actions occur at the price of loss of absorptive function and capacity. The viral infections of dogs attract the most attention to this aspect of the development of diarrhea, but the presence of any substance, which directly or indirectly results in the denudation of cells from a villus, will produce the same end result, diarrhea. Immunosuppressive drugs such as azathioprene, cyclophosphamide,

vincristine, and glucocorticoids can all cause severe atrophy of villi. Interestingly, some of these same compounds can facilitate epithelial cell renewal, with the type of end result being dose related and the undesirable result occurring only at high doses. Enterocytes located on villi can in the face of decreasing numbers alter their morphology in such a fashion as to cover or occupy a greater area over the basement membrane. Indeed, cells residing at the edges of ulcers and erosions are also stimulated to restore the gastrointestinal barrier by spreading out or migrating over vacant basal lamina. This is accomplished by means of an array of suitably anchored actin microfilaments, which permit cells to become taller, plumper, thinner, broader, or flatter. These individual cellular changes working in concert with changes in the shape and extension of individual villi brought about by threads of smooth muscle located in the villous lamina propria work to preserve epithelial barrier integrity. Failure to maintain this barrier can result in severe inflammation and deleterious changes.

Not unlike other organs of the body which are engaged in substantial activities, the mucosa of the gastrointestinal tract requires a persistent supply of oxygenated blood. Restriction of this flow can have disastrous consequences, including cell death and the complete sloughing of the gastrointestinal mucosa. The blood supply to the gastrointestinal tract has been previously discussed in detail and this flow is controlled by the central nervous system by means of sympathetic and parasympathetic nerves and also by the enteric nervous system, autoregulation, and sensory neurons contiguous to local blood vessels. A variety of neurotransmitters are involved which can bring about either vasoconstriction (norepinephrine, adenosine triphosphate, neuropeptide Y) or vasodilation (calcitonin gene-related peptide and vasoactive intestinal peptide). Autacoids can also function in the control of flows and they include vasodilators such as nitric oxide, prostaglandin E_2 , prostacyclin, and histamine and vasoconstrictors such as leukotriene C_4 and Thromboxane A_2 . Autocoids arise from vascular endothelial cells, smooth muscle endothelial cells, mast cells, and platelets. It is important to appreciate that sensory nerves do play an important role in the regulation of gastrointestinal blood flow, indeed calcitonin gene-related peptide and vasoactive intestinal peptide, both of which cause vasodilation can be released from sensory nerves with stimulation. Calcitonin gene-related peptide is considered to be a major contributor to the development of increased gastric mucosal blood flow. This increase in blood flow is associated with the presence of increased levels of nitric oxide. Increased blood flow is associated with increased oxygen supply and is therefore thought to be connected with the protection of the gastrointestinal mucosa from injury.

When speaking of the gastrointestinal tract one naturally at the same time thinks of nutrition. However, one seldom considers water a nutrient, but it is essential for life and death will occur much more rapidly in its absence than with the absence of food. A very important function of the gastrointestinal tract is not only the absorption of water, but also the secretion of aqueous fluid. The fluxes of water balance strongly favor absorption and the consequences to any organism with the development of diarrhea are simple, dehydration, and electrolyte imbalance. The dehydration results from an increased secretion of fluid into the intestinal lumen or decreased absorption of fluid from the intestinal stream or a combination of both. In any event, a loss of

extracellular and intracellular fluid from the body follows. With regard to electrolyte imbalance, bicarbonate ions are lost in the diarrhea, which leads to an increase in the anion gap (difference between positive and negative charges in the body) and an accumulation of intracellular hydrogen ion with the development of acidosis. The increasing amounts of positively charged hydrogen ion cause the shedding and decrease in levels of intracellular potassium ion. The reduction in the level of potassium impairs the homeostasis of existing electrochemical gradients and the accumulation of intracellular hydrogen ion changes pH and accordingly alters the level of function of many physiologic enzymes. All of these changes work together collectively and in a synergistic fashion to compromise the functions of cells, tissues, organs and the system as a whole. As extracellular potassium levels increase, there is enhanced elimination of potassium. Hyperkalemia can provoke cardiac arrest.

Neoplasia is not a commonly occurring problem with laboratory canines and so will not be discussed in detail here, but can produce diarrhea as a result of obstruction-induced fluid secretion or the release from tumors of physiologically active substances like histamine, gastrin, or 5-hydroxytryptamine [9,12,22,23,41,61,73,80,81,171,204,304–307]. These chemical mediators can also cause changes which result in intestinal bacterial overgrowth, a reduction in normal absorptive surface area, or the exudation of proteins and lipids. Neoplasia is usually the result of exposure to agents which bring about altered states of proliferation of gastrointestinal mucosa. Mechanisms can involve hyperproliferation, promotion, and alterations of apoptosis.

Impairment of the flow of lymph as a result of inflammation or the presence of some advanced states of hypertrophy in the intestinal tract can lead to the development of diarrhea. Inadequate lymphatic drainage from the intestine secondary to right-sided heart failure can also lead to the development of diarrhea.

Constipation is the opposite of diarrhea, cannot be considered to be a protective activity and can present a significant toxicologic or pathophysiologic event [406]. Indeed, constipation is one of two major toxicologic or pathophysiologic conditions of the colon or large intestine, the other being diarrhea. Constipation is characterized by lack of defecation, reduced defecation, painful defecation, or the passage of dry or concrete-like feces. The presence of blood in the stool, resulting from mucosal irritation due to abrasion from the hardened intestinal stream is an additional not infrequent clinical sign. Constipation is usually a transient event, but if it becomes persistent or is irreversible, it is referred to as obstipation. Obstipation not uncommonly can be associated with the permanent loss of function and can lead to the development of a condition known as megacolon or toxic megacolon, in which case the body can begin to absorb toxic substances from the static fecal stream. The pathophysiology of this condition is not entirely understood, but does appear to be neurally-based, but could also be the result of impairment of smooth muscle (longitudinal and circular) function. Nerves that can be affected are those of the autonomic or enteric nervous systems. In cases where megacolon has developed no gross or histological findings are observed, but smooth muscle develops a weaker level of tone when compared to normal smooth muscle upon stimulation by acetylcholine, substance P, and cholecystokinin. It is a current feeling that megacolon possibly results

from a disturbance in the intracellular cascade of events that occur subsequent to occupancy of a membrane-bound receptor, resulting in weaker contractile activity.

Conditions or agents that can be associated with constipation are inflammation of colonic epithelium, nerve dysfunction, myenteric plexus dysfunction, dehydration, hypokalemia, hypocalcemia, hypercalcemia, hypothyroidism, nutritional secondary hyperparathyroidism, barium sulfate, phenothiazines, cholinergic antagonists, diuretics, and opioid agonists.

The production of mucus and fluid is a benchmark response of toxicity that can occur throughout the entire length of the gastrointestinal tract and is typically the result of increased levels of production of normal processes that have been previously described in detail. Both responses are protective as well as toxic in nature.

Enterocytes possess a variety of different types of receptors on their surfaces. Some of these different receptors appear to be specific for select toxins, such as that expressed by *vibrio cholera*. The reason for this is unknown at this time, but the disruption of intracellular signal transduction systems typically results in the development of a secretory diarrhea. This diarrhea is a function of not just fluid secretion by enterocytes, but also nerve-mediated alterations of intestinal motility.

The gastrointestinal tract is heavily invested with a nervous supply as previously described, including its own myenteric nervous system. Neural function in the gastrointestinal tract is very complex and involves more than simple sensation and the stimulation of motor activities. The loss, impairment or stimulation of motor function affects the efficiency of the digestive process as well as absorption. However, nerves are also intimately involved in the regulation of regional blood flow and the release of substances essential for proper digestion and absorption. Accordingly, neural damage can serve as a prelude the development of diarrhea and malabsorption.

There are a variety of enzymes in the luminal space of the gastrointestinal tract as well as in the mucosal brush border and the mucosal epithelial cells themselves. In the presence of injury or inflammation, even more enzymes can be added to the mix from cells such as neutrophils. These latter enzymes can include gelatinases, elastases, and collagenases, which can bring about major structural damage to the scaffolding of the gastrointestinal tract upon which the mucosal epithelium rests. In conditions of toxicity, enzymes can be activated or inhibited and these alterations can lead to the development of gastrointestinal pathology. As discussed previously, many enzymes are secreted in an inactive form, awaiting a proper set of conditions for activation. For example, reactive oxygen species are able to avert normal processes and are capable of prematurely converting inactive enzymes to active forms. This alternate pathway and avoidance of normal control mechanisms can predispose the gastrointestinal mucosa to enzymatic damage.

EXAMPLES OF SPECIFIC TOXIC AGENTS

In the final portion of this chapter, a variety of illustrative examples are provided which demonstrate toxicity to and the responses of the gastrointestinal tract to the presence of a variety of different toxic agents. While the list of causative substances

is by no means complete and the mechanisms of pathophysiology not all-inclusive, the wide variety of substances and responses serve to underscore the complexity of an organ system, which does not attract the attention of higher profile organs such as the heart, liver, hematopoietic, and CNS systems.

Arsenic-containing compounds when ingested are readily absorbed by the mucosal epithelium of the intestine [331,337]. Once this occurs, enterocytes can rapidly die and be shed into the intestinal lumen. If this accelerated loss of enterocytes is of sufficient magnitude that normal protective compensatory actions cannot permit the maintenance of a complete, intact integral epithelial cell barrier, then areas of exposed basement membrane on the villi will develop. This exposure will lead to an altered state of permeability on the villi, with movement of exudates, transudates, and blood from the circulation into the intestinal lumen. This significant influx of fluid into the intestinal creates distension of the lumen, which stimulates increased motor activity with associated pain and diarrhea. The normal replacement of cells is hampered by the persistent or lingering presence of arsenic and barring complications, the gastrointestinal epithelium will eventually be replaced by normal reparative processes. Arsenates work by competing with phosphate ions, uncoupling oxidative phosphorylation and disrupting the production of energy necessary for the performance of normal enterocyte functions and cellular homeostasis. Arsenites react with the sulfhydryl groups present in proteins thereby altering active sites and the necessary specific three-dimensional structures required for activity. For example, arsenites inhibit alpha-keto oxidases, which are involved in the oxidation of pyruvate. The function of lipoic acid is also impaired. Lipoic acid is an essential coenzyme for both pyruvic acid oxidase and alpha-glutaric acid oxidase. Elemental arsenic induces vasodilation and vascular endothelial damage and organic pentavalent arsenic compounds may interfere with the functions of vitamins B₁ and B₆.

We are all very familiar with the toxicity caused by exposure to lead as manifested in the central nervous, hematopoietic and skeletal systems [350]. The gastrointestinal toxicity of lead is based primarily upon its ability to inhibit various enzyme systems. It too has an affinity for thiol groups and is also capable of competing with zinc and calcium in zinc and calcium-dependent enzyme systems. Lead interferes with deoxyribonucleic acid transcription factors by binding to cysteine residues. The calcium-mediated exocytosis of neurotransmitters can also be interfered with by the presence of lead. Protein kinase C, a calcium-dependent enzyme system, which regulates a variety of cellular activities including the growth of cells, is also adversely impacted by the presence of lead. While acute exposures to lead can result in vomiting and abdominal pain, chronic exposures can result in the development of constipation, which is most likely secondary to reduced motility. The reduction in motility is most likely due to the competition of lead with calcium in the contractile process.

Heavy meals, in general, can exhibit general corrosive effects on the mucosal epithelium of the gastrointestinal tract. Iron, for example, can cause severe ulceration and erosion of the stomach lining with resulting dramatic losses of fluid into the gastrointestinal tract lumen. A general theme of toxicity for agents of this type appears to be corrosion and enzyme inhibition.

Cholera toxin, although not a xenobiotic, is still considered to be a classical gastrointestinal toxin [17,77,196,211,379]. A discussion of its mode of action is valuable from the perspective of illustrating the consequences of alteration of intracellular signaling pathways. Cholera toxin is composed of a catalytic peptide unit (A) and five smaller peptide units (B). The B peptides bind to carbohydrates on G_{M1} gangliosides on the surface of intestinal epithelial cells. After binding, peptide A is delivered into the cell via calveolar-mediated endosomal entry. Upon entry into the cytoplasm, peptide A is cleaved by breakage of a disulfide bond into two fragments, A_1 and A_2 . The A_1 fragment is the important catalytic component and is involved with the development of pathology. A_1 interacts with twenty kilodalton cytosolic proteins called ADP-ribosylation factors. The resulting ADP-ribosylation factor- A_1 complex catalyzes the ADP-ribosylation of a forty-nine kilodalton G-protein (G_{sa}), which upon binding with nicotinamide adenine dinucleotide (NAD) and guanosine triphosphate (GTP) leads to the generation of an activated G_{sa} . This activated G_{sa} now binds to and stimulates adenylate cyclase. The ADP-ribosylated G_{sa} is permanently in an active GTP bound state, which results in the persistent activation of adenylate cyclase. The activated adenylate cyclase generates high levels of intracellular cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). Cyclic AMP stimulates the secretion of chloride and bicarbonate ions into the lumen of the intestine along with associated sodium ions and water, however sodium and chloride reabsorption also appear to be inhibited as part of this pathogenesis. Cholera toxin also exerts effects on the enteric nervous system, with subsequent alterations of gut motility and additional changes in the mucosal transport of fluid and electrolytes. It is important to note that in this case because the intestinal epithelium remains intact and essentially competent, most absorptive processes remain functional and competent as does the barrier function.

Non-steroidal anti-inflammatory drugs (NSAIDs) in general are organic acids and local gastrointestinal tissue damage is caused as a result of the direct contact of dissolved drug with the gastric mucosa [43,68,98,215,228,245,252,262,287,302,311,375]. Non-steroidal anti-inflammatory drugs increase gastric cell wall permeability and can uncouple oxidative phosphorylation, which collectively or individually can result in impaired cellular homeostasis and the development of cellular edema and apoptosis. While all areas of the gastrointestinal tract may theoretically be susceptible to the development of NSAID-induced lesions, the most frequent sites of damage are the stomach and duodenum. Ulcers (peptic) are the classic lesion with associated blood, fluid, and protein loss into the gastrointestinal stream. In advanced cases, ulcers can even progress into perforations of the gastrointestinal wall. However, the most important mechanism associated with exposure to non-steroidal anti-inflammatory drugs and the development of subsequent pathology is the reversible inhibition of the enzyme cyclooxygenase-1 (COX-1). The inhibition of COX-1 by NSAIDs initiates the development of pathology by blocking the production of prostaglandins in gastric tissues, which results in the decreased production of mucus and bicarbonate, the increased production of acid and decreased flow of blood into the gastric mucosa. The presence of underlying *Helicobacter pylori* infection can potentially worsen the toxic profile. The inhibition of COX-2 activity, however, is generally associated with a

lessening of the adverse effects that can be brought about by a triggering of the cyclooxygenase cascade. NSAIDs that are specific for inhibition of COX-2 activity provide the most beneficial and least troublesome toxicologic profile.

The cyclooxygenase system (COX) is responsible for the synthesis and production of prostaglandins. Cyclooxygenases exist in two forms, COX-1 and COX-2, each of which possess both cyclooxygenase and hydroxyperoxidase activities. COX-1 is a constitutive, noninducible enzyme that is normally present in the stomach, intestine and platelets and functions in the synthesis of platelet aggregation agents and the regulation of regional blood flow, thereby providing gastrointestinal mucosal protection. In contrast, COX-2 is an inducible enzyme, whose activity increases in response to the presence of a variety of different inflammatory stimuli. Indeed, the prostanoids produced by COX-2 are a segment of the typical inflammatory response. In general, cyclooxygenase-mediated prostaglandin synthetic activity can be initiated by a variety of different stimulators, including physical trauma, chemical exposure, cell proliferation, and inflammation. Arachidonic acid is the substrate for the COX enzyme system and is generated when phospholipase A₂ splits out arachidonic acid from the phospholipid membrane of the cell. Cyclooxygenases produce the unstable intermediate prostaglandin (PGG₂), which is quickly hydrolyzed via the same enzyme to prostaglandin H₂ (PGH₂). PGH₂ is unstable and rapidly metabolized by tissue-specific isomerases to multiple prostanoids such as prostacyclin (PGI₂), thromboxane A₂ (TXA₂), prostaglandin D₂ (PGD₂), prostaglandin E₂ (PGE₂) and prostaglandin F_{2a} (PGF_{2a}). These prostanoids stimulate specific G-protein coupled receptors to induce various effects which range from protection of the gastrointestinal tract to the aggregation of platelets (thrombus formation). PGI₂ is associated with vasodilation and the inhibition of platelet aggregation, while PGD₂, PGE₂, and PGF_{2a} are associated with vasodilation and the potentiation of edema. TXA₂ causes the aggregation of platelets and vasoconstriction. It is worth noting that thromboxane A₂ may also be a mediator in the development of gastrointestinal food-allergen hypersensitivity. The COX-1-mediated production of prostacyclin PGI₂ and prostaglandin PGE₂ are considered to be protective to the gastrointestinal mucosa essentially because of their vasodilation induction properties and resulting ability to preserve or enhance mucosal blood flow and oxygen supply, while the initiation of COX-2 activity is judged to be deleterious.

Mycotoxins are secondary fungal metabolites that exert toxic effects on a variety of different systems and a broad spectrum of species [172,393]. These materials are typically found as contaminants in grains and grain-based products. There are a variety of different types of mycotoxins and we will speak of only one here, the trichothecenes. Trichothecenes are produced by *Fusarium spp.* and include such compounds as deoxynivalenol (vomitoxin), T-2 toxin, and diacetoxyscirpenol. Gastrointestinal toxicity is typically manifest as vomiting, bloody diarrhea, dehydration and weight loss. Trichothecenes in general inhibit protein synthesis, which eventually causes cell death. Deoxynivalenol can delay gastric emptying and intestinal motor activity possibly through a serotonin-mediated pathway. The actively dividing tissues of the gastrointestinal tract are most susceptible to the actions of T-2. It is important to note that even for certified feed, the presence or levels of mycotoxins are not routinely determined by analysis.

The pyrimidine analog 5-fluorouracil (5-FU) is a halogenated derivative of pyrimidine and an antineoplastic agent of the antimetabolite class [174,280]. 5-FU requires enzymatic conversion to the nucleotide in order to exert its cytotoxic activity. Incorporation of 5-FU into both RNA and DNA occurs, however the relevance and importance of the incorporation into DNA is unclear because normal excision repair processes might well remove the halogenated moiety before any toxic consequence can be elicited. Alternatively, incorporation of 5-FU into RNA causes toxicity as a result of the exertion of major effects on both the processing and functions of RNA thereby inhibiting cell division. Accordingly, the rapidly dividing cells of the gastrointestinal tract are ideal targets for these types of compounds.

Steroids resemble closely the toxicity manifest by NSAIDs. This is because steroids inhibit the function of phospholipase A₂, which generates arachidonic acid, the substrate for the cyclooxygenase enzymatic system described above. In the lack of presence of substrate an adequate supply of prostacyclin and prostaglandins is not able to be sustained. Subsequently, the beneficial and protective effects of prostaglandins are missing, which leaves the gastrointestinal mucosa susceptible to injury and compromise.

The ingestion of a variety of plants, plant materials and plant extracts are followed by the development of nausea, vomiting, diarrhea, and bloody diarrhea. The most common mechanism by which gastrointestinal toxicity occurs is direct irritation of the gastric or intestinal mucosa. Another mechanism for plant-induced gastrointestinal toxicity is antimitosis. Colchicine is probably the best known example of this mechanistic type of toxicity. The antimitotic action is brought about by the blockage of formation of microtubules and subsequent failure of the formation of a competent mitotic spindle. Finally, there is a group of compounds in a variety of plants, glycoproteins, which can interact with select carbohydrate moieties present on the membranes of mucosal epithelial cells. Once binding is complete, the function of the brush border on the luminal surfaces of enterocytes is altered and a nonspecific inhibition of all absorption gradually develops. One of these glycoproteins is a group of compounds referred to as lectins. Upon entry into cells themselves, lectins inhibit protein synthesis, causing cell death.

A group of classic gastrointestinal toxins is that of the acetylcholinesterase inhibitors. Acetylcholine is a neurotransmitter present at synaptic and neuroeffector endings of cholinergic motor and secretomotor neurons of the enteric nervous system. Inhibition of the enzyme acetylcholinesterase leads to an accumulation of acetylcholine at synapses or effector sites and resultant persistent stimulation. Increased motor activity is the result of the interaction of acetylcholine with M₃ muscarinic receptors and the increase in gastric and intestinal secretions the result of stimulation of both M₁ and M₃ muscarinic receptors. Inhibition of acetylcholinesterase activity causes the secretion of large volumes of fluid and amounts of electrolytes into the gastrointestinal tract, with the development of a profuse watery diarrhea accompanied by a severe cramping pain.

Sucralfate is an oral antiulcer agent that acts by forming a protective barrier over the site of an ulcer and is a complex of sucrose octasulfate and polyaluminum hydroxide. In the local environment of the stomach, polymerization and cross-linking

occur, which results in the formation of a sticky, yellow-white gel that combines with proteinaceous exudates in the stomach to form an adherent barrier preventing the contact of gastric acid with the lesion compromising the integrity of the mucosal barrier. However, continued exposure can result in the slow release of aluminum into the system and the development of constipation by a mechanism that is unclear.

Ethanol is not uncommonly used as a vehicle or excipient in a variety of different concentrations and along with other alcohols can directly induce toxicity on the mucosal epithelial cell of the gastrointestinal tract. This toxicity is characterized by the formation of hemorrhagic erosions in the gastric mucosa and is chiefly the result of a disordering of the lipid bilayer of the cell membrane, which results in altered membrane fluidity and subsequently cell damage, loss of cellular homeostasis, and cell death. Vascular injury can also develop concurrently as a result of direct cytotoxicity, which of itself then leads to the degranulation of mast cells, release of leukotrienes, and enhanced mucosal permeability. In the presence of a sound oxygen supply and good flow of blood, restitution of the mucosal epithelial cells can occur on a timely basis. Continued exposure to ethanol can result in the increased production of various growth factors such as epidermal growth factor, which possibly protect the gastric mucosa from injury by stimulating the rate of cell proliferation, but also generate susceptibility to the possible development of neoplasia. Exposure to ethanol can inhibit the secretion of bicarbonate ion, impair the synthesis of mucus, increase luminal sodium ion concentration, enhance the permeability of the mucosa, permit the back-diffusion of hydrogen ion, and in a dose-response fashion deplete intracellular stores of glutathione. At high concentrations, exposure to ethanol can be associated with increased levels of synthesis of prostacyclin and prostaglandins, which possibly offer protective effects to the gastrointestinal mucosa after the ethanol-induced injury.

GASTROINTESTINAL SYSTEM FUNCTION AND CIRCADIAN RHYTHMS

Chronobiology is a field of biology that examines periodic (cyclic) phenomena in living organisms and the adaptation of these organisms to solar- and lunar-related rhythms. These cycles are known as biological rhythms [29,31,65,66,394]. The term “chronobiology” comes from the ancient Greek χρόνος (chrónos, meaning “time”), and biology, which pertains to the study, or science, of life. The related terms “chronomics” and “chronome” have been used in some cases to describe either the molecular mechanisms involved in chronobiological phenomena or the more quantitative aspects of chronobiology, particularly where comparison of cycles between organisms is required. Chronobiological studies include but are not limited to comparative anatomy, physiology, genetics, molecular biology, and behavior of organisms within biological rhythms mechanics. Other aspects include development, reproduction, ecology, and evolution.

The variations of the timing and duration of biological activity in living organisms occur for many essential biological processes. These occur (1) in animals

(eating, sleeping, mating, hibernating, migration, cellular regeneration, etc.), (2) in plants (leaf movements, photosynthetic reactions, etc.), and (3) in microbial organisms such as fungi and protozoa (biochemical and metabolic processes). They have even been found in bacteria, especially among the cyanobacteria (e.g., blue-green algae). The most important rhythm in chronobiology is the circadian rhythm, a roughly 24-hours cycle demonstrated in the physiological processes in all these organisms. The term “circadian” comes from the Latin *circa*, meaning “around” and *dies*, “day,” meaning “approximately a day.” It is regulated by circadian clocks.

Knowledge of these patterns (for the specific species in question) facilitates optimal drug delivery in terms of bioavailability and achieving better specificity in delivery.

The circadian rhythm can further be broken down into routine cycles during the 24-hour day:

- Diurnal, which describes organisms active during daytime
- Nocturnal, which describes organisms active in the night
- Crepuscular, which describes animals primarily active during the dawn and dusk hours (e.g., skunks, white-tailed deer, woodchucks)

While circadian rhythms are defined as being endogenously regulated, other biological cycles may be regulated by exogenous signals. In some cases, multi-trophic systems may exhibit rhythms driven by the circadian clock of one of the members (which may also be influenced or reset by external factors such as light). The endogenous plant cycles may regulate the activity of the bacterium by controlling availability of plant-produced photosynthate.

Many other important cycles are also studied, including:

- Infradian rhythms, which are cycles longer than a day, such as the annual migration or reproduction cycles found in certain animals or the human menstrual cycle.
- Ultradian rhythms, which are cycles shorter than 24 hours, such as the 90-minute REM cycle, the 4-hour nasal cycle, or the 3-hour cycle of growth hormone production.
- Tidal rhythms, commonly observed in marine life, which follow the roughly 12.4 hour transition from high to low tide and back.
- Lunar rhythms, which follow the lunar month (29.5 days). They are relevant e.g., for marine life, as the level of the tides is modulated across the lunar cycle.
- Gene oscillations—some genes are expressed more during certain hours of the day than during other hours.

Within each cycle, the time period during which the process is more active is called the acrophase. When the process is less active, the cycle is in its bathyphase or trough phase. The particular moment of highest activity is the peak or maximum; the lowest point is the nadir. How high (or low) the process gets is measured by the amplitude.

All eukaryotic organisms ranging from insects to mammals have over time developed behavioral, physiological and/or molecular biological and biochemical processes that have a particular rhythm that is synchronized to time and time periods

within each day or even over a period of days. These processes enable an organism to adapt to its own special environment most optimally, quickly and with greater facility. Again, these circadian oscillations or rhythms are endogenous in nature to each organism, specific to each organism and most commonly exhibit a periodicity or single cycle length of 24 hours and are responsive to and can be adjusted by the stimulus of light. Succinctly, the study of the time-related periodic changes in the susceptibility, responses and sensitivity of organisms expressed as the pharmacology, pharmacokinetics, pharmacodynamics, and adverse effects of drugs, xenobiotics, toxins, chemicals, etc. is designated as chronotoxicology, chronopharmacodynamics, chronopharmacokinetics, or chronopharmacology.

It is important to recognize that these temporal rhythms are driven by circadian (24-hour time period) or solar time cycles, which are in turn based upon periodic photo-stimuli received from sunlight and potentially further influenced by other local time signals.

The biological rhythms of living organisms have been well-known for several decades as a result of numerous and various reports in the published literature of cell biology, physiology, molecular biology, biochemistry, and pharmacology. Temporal variations can and do simply explain why the same xenobiotics etc., regardless of origin, do not induce the same efficacy or other effects in a living organism, if it is administered at different times of the day or at different seasons of the year. Rhythmic or circadian changes in the sensitivity or susceptibility to xenobiotics were actually first reported as far back as 1958 in experiments with mice. A few years later it was demonstrated that German cockroaches (*Blatella germanica*) exhibited a rhythmic susceptibility over a 24-hour period of time to a standard dose of potassium cyanide, when organisms were exposed to the toxin at different times within the day.

The mammalian circadian system is organized in a prioritized manner with a highly important centrally located pacemaker within the suprachiasmatic nucleus (SCN) of the hypothalamic region of the brain. This structure is located proximal to the optic chiasm and receives transmitted light through the eyes within the wavelength range of 460–480 nm. The reception and processing of this light is through non-visual photosensitive retinal ganglion cells, which contain the substance melatonin and are located within the retino-hypothalamic tract of the brain. This center controls and synchronizes the circadian receivers or oscillators in millions of cells that are located all over the body. These cells themselves are not responsive to light, but do respond to various neural and neuro-humoral signals from the SCN in the brain. Major timing signals in the synchronization of many of these peripheral clocks include sleep and activity periods and feeding and fasting cycles. This has suggested to researchers in the field that the temporal coordination of metabolism and cell proliferation is a major effort of the mammalian timing, circadian, circannual, or other periodic system. Taken together, the complete chronological biological system directs and controls precisely timed events, which sustain life by regulating physiological function of the cardiovascular, hemodynamic, metabolic, nervous, digestive, immune, reproductive, and endocrine systems [4,10,29,50,56,65,100,110,150,226,244,268,325]. Rhythmic fluctuations in absorption, distribution, metabolism and excretion regulate the detoxification of toxic substances, drug toxicity, and drug efficacy.

This watch-like control of drug and xenobiotic catabolism and detoxification provides the molecular and biochemical basis for the dosing time-dependence of both drug toxicities and drug efficacies. The inactivation of noxious food components by intestinal, hepatic, and renal detoxification systems is among the processes regulated in a circadian fashion. Many of these chronologic processes are further regulated at the genetic level by actual clock genes (not discussed here), which establish basic transcriptional and translational feedback oscillation loops under the control of such entities within the body as *Per* 1–3, *Cry* 1,2, *Rev-erb α* , *Rev-erb β* , *Ror α* , *Ror β* and cAMP response element-binding protein (CREB) along with the circadian locomotor output cycles involving ARNT-like protein 1 (CLOCK/BMAL 1) heterodimers. Knowledge and understanding of these clock-based processes continues to grow and expand and this information can be used for improving or designing therapeutic treatment regimens.

It has not been an uncommon finding that some studies in the area of chronotoxicology are also susceptible to seasonal variability. Reports have shown that some circadian peak times of susceptibility found during one season may be totally different from that observed in a different season.

TIMED RHYTHMS AND THE HEPATIC SYSTEM

Over the years a variety of agents have been reported in the literature as being hepatotoxic and the solvent carbon tetrachloride has been a popular agent that has been studied [31,52,209,269,394]. After a single dose exposure of carbon tetrachloride, centrilobular necrosis and lipidosis occurs in the livers of all animals. Mitochondrial damage can be observed by 5–6 hours after exposure. Other lesions develop by 12 hours post exposure and severe hepatic necrosis has developed by 24 hours post exposure. Although trichloroethylene is a well-known and highly-characterized hepatotoxin, it is also a potent neurotoxin. For example, if trichloroethylene is administered to rats via the intra-peritoneal route at four different time points within a day and toxicity assessed by evaluating muscle tone, it was determined that the time of the most significant neurotoxic effect of trichloroethylene was early in the active phase (21:00 hour; nighttime). Interestingly, the plasma concentrations of trichloroethylene and two of its major metabolites are found to be at minimal levels at 21:00 hour (nighttime). Accordingly it appears that neurotoxicity of trichloroethylene is accentuated when hepatic elimination is at its lowest level of activity. Studies have demonstrated that the circadian susceptibility of animals to hepatotoxicity has been found to be at least in part dependent on the timing of toxin uptake and metabolism. Similar correlations with chronotoxicity have been found in studies with the agents chloroform, trichloroethylene, 1,1-dichloroethylene, and the drug acetaminophen. Rats have been found to be most susceptible to chloroform exposure when the administration is performed within a 2-hour period after the initiation of their active period (nighttime) (21:00 hour). The lowest level of susceptibility was observed when exposure was at 09:00 hour (daytime). Even though rats exposed to chloroform at both 21:00 hour and 09:00 hour revealed depressed levels of glutathione, hepatic

levels of glutathione were found to be more significantly depressed at 21:00 hour than at 09:00 hour. If the serum activities of glutamic-pyruvic-transaminase, isocitrate dehydrogenase, ornithine-carbamyl-transferase, and sorbitol dehydrogenase were measured in rats after exposures to carbon tetrachloride at eight different time points within a day, it was revealed that significant increases (in some cases approaching 400%) were observed with the administration of carbon tetrachloride at 18:00 hour or 20:00 hour, but remained unchanged if exposure was at 10:00 hour.

TIMED RHYTHMS IN ABSORPTION

Absorption can be most simply defined as the transfer of a test article from the site of its administration into the systemic circulation [31,368]. Generally, the most important pharmacokinetic parameters involved in the process of absorption are the maximal blood/serum/plasma concentration ($C_{\max}$), the time to attain the $C_{\max}$ value ($t_{\max}$), the absorption rate constant (k_a), and the area under the curve (AUC). The AUC value is an estimate of the amount of drug absorbed and serves as an extremely useful tool to compare the bioavailabilities of different formulations, but also to make comparisons from the pharmacokinetic perspective using ratios of AUC values from an intravenous administration compared to other routes of administration. There are a variety of factors that can affect absorption and they include the physicochemical characteristics of the test article itself, the dosage form, formulation, and biological function. Any one of these factors has the potential to involve diurnal oscillations and accordingly subsequent changes in the pharmacokinetic parameters with time.

Membrane transporters can be major determinants of the pharmacokinetic, safety, and efficacy profiles of drugs. This presents several key questions for drug development, including which transporters are clinically important in drug absorption and disposition, and which *in vitro* methods are suitable for studying drug interactions with these transporters. A classification of drug transporters is represented in [Table 6.1](#).

In addition, what criteria should trigger follow-up clinical studies, and which clinical studies should be conducted. Overall, it is advised that the timing of transporter investigations should be driven by efficacy, safety, and clinical trial enrolment questions (e.g., exclusion and inclusion criteria), as well as a need for further understanding of the absorption, distribution, metabolism and excretion properties of the drug molecule, and information required for drug labeling. Membrane transporters can be major determinants of the pharmacokinetic, safety, and efficacy profiles of drugs. This presents several key questions for drug development, including which transporters are clinically important in drug absorption and disposition, and which *in vitro* methods are suitable for studying drug interactions with these transporters. In addition, what criteria should trigger follow-up clinical studies, and which clinical studies should be conducted. The general feeling these days is that the timing of transporter investigations should be driven by efficacy, safety and clinical trial enrolment questions (e.g., exclusion and inclusion criteria), as well as a need for further understanding of the absorption, distribution, metabolism, and excretion properties

Table 6.1 Classification of Drug Transporters^a

Transporter Family	Family Member	Gene Name
Organic cation transporter (OCT)	hOCT1	SLC22A1
	hOCT2	SLC22A2
	hOCT3	SLC22A3
Organic cation/carnitine transporter (OCTN)	OCTN1	SLC22A4
	OCTN2	SLC22A5
	OCTN3	SLC22A21
	CT2	SLC22A16
Organic anion transporter (OAT)	OAT1	SLC22A6
	OAT2	SLC22A7
	OAT3	SLC22A8
	OAT4	SLC22A11
	OAT5	SLC22A10
	OAT6	SLC22A20
Organic anion transporter polypeptides (OATPs)	URAT1	SLC22A12
	OATP1C1	SLC01C1
	OATP1B1	SLC01B1
	OATP1A2	SLC01A2
	OATP1B3	SLC01B3
	OATP2A1	SLC02A1
	OATP2B1	SLC02B1
	OATP3A1	SLC03A1
	OATP4A1	SLC04A1
	OATP4C1	SLC04C1
Peptide transporter (PEPT)	OATP5A1	SLC05A1
	OATP6A1	SLC06A1
	PEPT1	SLC15A1
	PEPT2	SLC15A2
Monocarboxylate transporters (MCTs, sMCTs)	PHT1	SLC15A4
	PHT2	SLC15A3
	MCT1	SLC16A1
	MCT2	SLC16A7
	MCT3	SLC16A8
	MCT4	SLC16A3
Nucleoside transporters (CNTs, ENTs)	SMCT1	SLC5A8
	SMCT2	SLC5A12
	CNT1	SLC28A1
	CNT2	SLC28A2
	CNT3	SLC28A3
	ENT1	SLC29A1
	ENT2	SLC29A2
	ENT3	SLC29A3
Bile acid transporters	ENT4	SLC29A4
	NTCP	SLC10A1
	ASBT	SLC10A2
	BSEP	ABCB11
	OST- ∞	—
Multidrug resistance protein (MDR)	OST- β	—
	MDR1	ABCB1

(Continued)

Table 6.1 (Continued) Classification of Drug Transporters

Transporter Family	Family Member	Gene Name
Multidrug resistance–associated protein (MRP)	MRP1	ABCC1
	MRP2	ABCC2
	MRP3	ABCC3
	MRP4	ABCC4
	MRP5	ABCC5
	MRP6	ABCC6
	MRP7	ABCC10
	MRP8	ABCC11
	MRP9	ABCC12
Breast cancer resistance protein (BCRP)	BCRP1	ABCG2

^a References where the human chromosome locus can be found.

of the drug molecule, and information required for drug labeling. Unfortunately, there is no data available on the connection between transporter activity and biological rhythms.

A large number of drugs, regardless of their route of administration (exclusive of intravenous), are absorbed via the simple partition process of passive diffusion. Drugs such as diclofenac, ketoprofen, nifedipine, prednisolone, propranolol, salicylic acid, valproic acid, and many others are known to be absorbed to a greater degree and faster if administered in the morning to a human as a single dose in a nonexotic and non-sustained-release formulation. These reports demonstrate minimally the potential importance of the time of the day that administration is performed and the determination of bioavailability [30,31,54,87,224,340].

To be effectively absorbed, any solid pharmaceutical formulation must first be broken down into very small particles, which then need to be solubilized in an aqueous milieu at the site of absorption to initiate this passive diffusion process and absorption.

Medicines such as gentamicin, midazolam, and acetaminophen have demonstrated no temporal correlation when administered orally. While it is true that the mechanisms associated with morning administration and differences in bioavailability have never been adequately characterized in detail, it is fair to state that an adequate understanding of the physicochemical properties of a test article coupled with an appreciation of the circadian oscillations of the gastrointestinal tract can explain the results.

If one looks at the $C_{\max}$ and $t_{\max}$ values for indomethacin, phenylbutazone, hydrochlorothiazide, and furosemide after single oral administrations at 08.00 hour and 20.00 hour, no time-related change was observed for the $C_{\max}$ value of furosemide and hydrochlorothiazide, but the $t_{\max}$ values were found to be shorter for the evening administration. The furosemide and hydrochlorothiazide were administered as solutions and the indomethacin and phenylbutazone were administered as suspensions of powder. However, the phenylbutazone and indomethacin demonstrated greater $C_{\max}$ values and shorter $t_{\max}$ values at the 20.00 hour administration. The most significant diurnal variations were obtained with drugs given as suspensions, suggesting that the rate of solubilization of the two drugs is higher in the evening.

DIURNAL RHYTHMS AND THE GASTROINTESTINAL TRACT

It is interesting to note that the circadian oscillatory gastrointestinal absorptive behavior in many studies is eliminated if the drugs are administered in a sustained-release form or as a rectal suppository. However, it has been shown for indomethacin and other drugs (e.g., theophylline) that some sustained-release formulations do exhibit variations in time for oral absorption, but this is different from the diurnal pattern of the immediate-release formulation of the drug. Reports also suggest that the rate of drug solubilization from the administered form of the drug is a determining factor in the temporal pattern of absorption for many drugs [31,45,97,101,124,126,138,182,206,208,209,214,218,224,265–267,297,308,348,366,387,391].

In cases of oral administration, whether by powder, liquid, suspension, capsule, tablet, slurry, etc. a number of factors can influence subsequent plasma levels. These factors include but are not limited to gastrointestinal transit times and the rate of gastric emptying. While there are drugs that act on the stomach lining, in general test articles are not absorbed in the stomach and the absorption process only begins when they enter the intestine and such biological functions as gastric emptying and gastrointestinal transit times are indeed affected by circadian rhythms, which then in turn affect the rate of absorption of a material. Not surprisingly, for many drugs the bioavailability does not change with either a daytime or nighttime administration. However, dosage in the evening may be a better approach for drugs exhibiting a lower threshold of toxicity, because side effects are associated with plasma levels and gastric emptying times are slower in the evening.

Studies involving the assessment of gastric diurnal toxicology using gastric mucosal integrity have revealed that there is indeed a time-related damage component caused by the well-characterized aspirin and other nonsteroidal anti-inflammatory (NSAID) agents. Although still somewhat controversial, it appears that the nighttime administration of these types of agents are better tolerated than the daytime administration of the same. In humans, using endoscopic visualization, it was determined that the administration of 1300 mg of aspirin at 08:00 hour versus 20:00 hour resulted in the development of almost 40% fewer gastric mucosal lesions if the medicine was administered in the evening. In a study utilizing osteoarthritic patients, it was discovered that the administration of a sustained-release formulation of indomethacin at 08:00 hour resulted in a significantly greater number of undesirable side effects than if the medication was administered in the evening at 20:00 hour.

Drugs that are completely disintegrated or dissolved in the stomach are completely emptied into the intestine in a liquid phase that does not display a significant circadian variation in absorption if they are compared to drugs that are ingested with solids. It is well-known that the ingestion of a solid meal delays the absorption of drugs because of the inhibitory effects of solids on gastric emptying. Since gastric emptying occurs more slowly in the evening as compared to the morning, this delay is amplified in the evening. The absorption of enteric or delayed release forms are even more delayed with concurrent meal ingestion. The presence of food in the stomach inhibits the gastric migrating motor complex waves that move solids such as enteric coated capsules out of the stomach and into the small intestine,

where their dissolution begins. The speed of migrating motor complex waves is slower at night. If for some reason there is a decreased frequency of migrating motor complex waves, gastric residence times are increased. As one can see there are a number of different factors in play here and it is entirely possible that a sustained-release form administered in the morning on a full stomach may not be absorbed during the day.

The rhythmic motor patterns of the gastrointestinal tract (stomach and intestines) strongly influence the absorption and distribution of orally delivered medicaments. Test articles that are administered via the oral route must exit the stomach before the process of absorption occurs. The transfer of drugs into the small intestine is related to the rate of gastric emptying and that itself is influenced by a number of factors including the presence of food, the absence of food, whether the food is in liquid form or solid form, the percent of amount of protein, the percent or amount of carbohydrate, the percent of amount of lipid, body posture, activity level, gender, age, and circadian oscillations. In the presence of food, gastric motor activity is pretty much dominated by caudally-directed peristaltic contractions. These contractions occur as a result of the synergistic activity of muscles in the wall of the stomach, which as a result of their activity grind solids to a very small particle size, which in turn permits facile passage from the stomach into the small intestine. Liquids are emptied from the stomach more easily. Liquids exit the stomach generally according to first-order kinetics and digestible solids exit the stomach according to zero-order kinetics. Indigestible solids are not able to exit the stomach as liquids and digestible solids do, and are emptied from the stomach by the migrating motor complex, which is subject to rhythmic oscillations of various durations, frequencies, and periods. In humans, gastric emptying rates were significantly different, when subjects were fed at two different time points. Subjects consuming food at 20:00 hour demonstrated significantly slower gastric emptying rates for the same meal administered to the same subjects as 08:00 hour.

Gastric acid secretion is stimulated by a number of different routes and pathways, many of which can be independently blocked by various inhibitors. Without stimulation, and in the presence of fasting, gastric acid is secreted in low amounts to maintain a pH in the stomach within the range of 0.8 and 2.5 for a 24 hour period of time. Gastric acid output (acid and various fluids) is highest in the evening and lowest during the morning hours in the absence of the presence of food. The activities and kinetic parameters of many enzyme systems of the intestinal lining have also been shown to vary with the time of the day. Both circadian oscillations and the presence of both liquid and solid food play significant synergistic role in regulation of gastric emptying.

Delayed intestinal absorption rates may result in enhanced first pass effects. However, administration in the morning hours can be a desirable course of action because of a more rapid onset-of-action due to increased gastric emptying activity.

Motility of the gastrointestinal tract and biliary secretion are two physiologic activities that involve circadian rhythms and enhance the rate of drug dissolution. In humans maximal amounts of bile acids are reported to be secreted between the times of 08:00 hour and 14:00 hour, which correspond to the actual period of the day of

maximal absorption. The synthesis of bile acids in humans also follows a circadian oscillation with the maximal synthetic activity being in the morning and the nadir of synthetic activity being late in the afternoon and evening. Very similar patterns of bile acid synthesis and bile flow have been described in rats, where the highest period of activity was observed at midnight and the nadir of activity at noon, during the sleep period. In short, the higher load of bile acids secreted into the small intestine and the greater rate of gastric emptying and intestinal motility during the morning hours parallel the time increase in the absorption parameters of most drugs in humans.

Cardiac output to the gastrointestinal tract, blood flow and blood pressure follow a pattern of circadian rhythmic change which support the variations of different degrees of absorption of drugs from the gastrointestinal tract. In studies with rats it was observed that peak concentrations of radioactivity in the gastrointestinal tract were at their maximum at 03:00 hour and 21:00 hour (activity periods) and their nadir during the day time.

BIOLOGICAL RHYTHMS AND MIXED FUNCTION OXIDASES

Drug metabolism is the metabolic breakdown of drugs by living organisms, usually through specialized enzymatic systems [29–34,123,136,148,202,230,236,263,273,286,296,301,316,364,410]. More generally, xenobiotic metabolism (from the Greek *xenos*, “stranger” and *biotic*, “related to living beings”) is the set of metabolic pathways that modify the chemical structure of xenobiotics, which are compounds foreign to an organism’s normal biochemistry, such any drug or poison. These pathways are a form of biotransformation present in all major groups of organisms and are considered to be of ancient origin. These reactions often act to detoxify poisonous compounds (although in some cases the intermediates in xenobiotic metabolism can themselves cause toxic effects). The study of drug metabolism is called pharmacokinetics.

The metabolism of pharmaceutical drugs is an important aspect of pharmacology and medicine. For example, the rate of metabolism determines the duration and intensity of a drug’s pharmacologic action. Drug metabolism also affects multidrug resistance in infectious diseases and in chemotherapy for cancer, and the actions of some drugs as substrates or inhibitors of enzymes involved in xenobiotic metabolism are a common reason for hazardous drug interactions. These pathways are also important in environmental science, with the xenobiotic metabolism of microorganisms determining whether a pollutant will be broken down during bioremediation, or persist in the environment. The enzymes of xenobiotic metabolism, particularly the glutathione S-transferases are also important in agriculture, since they may produce resistance to pesticides and herbicides.

Drug metabolism is divided into three phases. In phase I, enzymes such as cytochrome P450 oxidases introduce reactive or polar groups into xenobiotics. These modified compounds are then conjugated to polar compounds in phase II reactions. These reactions are catalyzed by transferase enzymes such as glutathione S-transferases. Finally, in phase III, the conjugated xenobiotics may be further processed, before being recognized by efflux transporters and pumped out of cells.

Drug metabolism often converts lipophilic compounds into hydrophilic products that are more readily excreted.

Most xenobiotics and drugs are eliminated from the body by drug metabolism, which involves a variety of different chemical reactions catalyzed by a wide spectrum of enzymatic systems (e.g., P-450 monooxygenases) that are for the most part located in the mammalian liver. The enzymes performing these metabolic changes exist in a variety of different isomeric forms, each with different kinetic properties and different substrate requirements. There are a number of reviews in the literature, which describe the circadian oscillations of hepatic metabolism.

Phase I reactions are those that introduce a polar reactive group into lipophilic drugs or xenobiotics. In most cases this group becomes the site for conjugation during phase II reactions. Such reactions include microsomal mono-oxygenations, cytosolic and mitochondrial oxidations, co-oxidations in prostaglandin synthetase reactions, reductions, hydrolysis reactions, and epoxide hydration. The products of phase I reactions may be potent electrophiles that can be conjugated and detoxified in phase II reactions or which may react with nucleophilic groups or cellular constituents, thereby showing greater toxicity than the parent compound. The epoxidation of polycyclic aromatic hydrocarbons is one example of a typical phase I reaction.

Cytochromes P450 (CYPs) are proteins of the superfamily containing the heme group as a cofactor and, therefore, are hemoproteins. CYPs use a variety of small and large molecules as substrates in enzymatic reactions. They are, in general, the terminal oxidase enzymes in electron transfer chains, broadly categorized as P450-containing systems. The term “P450” is derived from the spectrophotometric peak at the wavelength of the absorption maximum of the enzyme (450 nm) when it is in the reduced state and complexed with carbon monoxide. CYP enzymes have been identified in all domains of life: animals, plants, fungi, protists, bacteria, archaea, and even in viruses. However, they are not omnipresent; for example, they have not been found in *Escherichia coli*. More than 21,000 distinct CYP proteins are known. Most CYPs require a protein partner to deliver one or more electrons to reduce the iron (and eventually molecular oxygen). Based on the nature of the electron transfer proteins, CYPs can be classified into several groups:

- Microsomal P450 systems, in which electrons are transferred from NADPH via cytochrome P450 reductase (variously CPR, POR, or CYPOR). Cytochrome b5 (cyb5) can also contribute reducing power to this system after being reduced by cytochrome b5 reductase (CYB5R).
- Mitochondrial P450 systems, which employ adrenodoxin reductase and adrenodoxin to transfer electrons from NADPH to P450.
- Bacterial P450 systems, which employ a ferredoxin reductase and a ferredoxin to transfer electrons to P450.
- CYB5R/cyb5/P450 systems, in which both electrons required by the CYP come from cytochrome b5.
- FMN/Fd/P450 systems, originally found in *Rhodococcus* species, in which a FMN-domain-containing reductase is fused to the CYP.
- P450 only systems, which do not require external reducing power. Notable ones include thromboxane synthase (CYP5), prostacyclin synthase (CYP8), and CYP74A (allene oxide synthase).

Table 6.2 Summary of the Most Common Human Cytochrome P450 Genes and the Proteins They Encode

Family	Members	Names	Function
CYP1	3 subfamilies, 3 genes, 1 pseudogene	CYP1A1, CYP1A2, CYP1B1	Drug and steroid (especially estrogen) metabolism, benzo[a]pyrene (forming (+)-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide)

Humans have 57 genes and more than 59 pseudogenes divided among 18 families of cytochrome P450 genes and 43 subfamilies. A summary of the human cytochrome P450 genes and the proteins that they encode are listed in [Table 6.2](#).

Of these enzymes and enzymatic systems referred to above, oxidative reactions are the most common, the most frequently studied and the most important. The spectrum of substrates involved and the biological consequences of the products are wide. Indeed, oxidation reactions are in a large number of cases the rate limiting process in the clearance of a drug from the body and they are the central point of consideration for many drug interactions that are of interest from a clinical perspective. Initial reports regarding the circadian oscillations of hepatic microsomal metabolism in the mouse and rat appeared in the late 1960s. In these reports in the literature the values of oxidase activities were compared for the time periods of 22:00 hour–02:00 hour and 10:00 hour–14:00 hour, and the highest levels of activities were found in the period 22:00 hour–02:00 hour and the lowest values were found in the period 10:00–14:00 hour. Utilizing purified microsomes isolated from rat livers, the oxidase activities for different for different substrates was determined it was determined that the activities of aminopyrene de-ethylases and aniline hydroxylase were found to be higher at 21:00 hour than at 09:00 hour. However these researchers also found that the activity levels of testosterone 7 α - and 6 β -hydroxylases and the P-450 reductase were lower in the evening hours. When results for activity levels for testosterone 16 α -hydroxylase were investigated, no diurnal rhythm was found and there was also no circadian effect on total amounts of cytochrome P-450 and protein. Interestingly, a very significant difference was found when day versus night time levels of the microsomal concentration of fatty acids were compared. At 09:00 hour in the morning the levels of various lipids were found to be much higher than in the evening, with the exception that arachidonic acid levels exhibited measurable amounts at 21:00 hour. The activity of each oxidase studied is mediated selectively by at least one cytochrome P-450 isoenzyme. The changes in activities of the various cytochromes P-450 in time with exposure to differing substrates supports the contention that there are indeed diurnal variations in enzymatic kinetic parameters as well as amounts of some P-450 isoenzymes. The cytochromes P-450 isozymes IIB1, IIC11, and IIC6 were examined for temporal oscillatory behavior in rat liver microsomes using the Western blotting technique, selectivity for enantiomers and the regiospecificity of testosterone hydroxylases at the 2 α , 6 β ,16 α , and 16 β

positions. Significant differences were observed in the temporal rhythms of total P-450 amounts for the various isoenzymes. Similarly, typical variations in time were found in the rates of various hydroxylations of testosterone at different sites on the core molecule (e.g., carbons 2, 6, and 16) or on the same carbon atom with products of differing conformation being generated (2α or 2β and 16α , or 16β). However, correlation was found to be poor between oxidase activities and the total amount of microsomes. A lot of work remains to be performed in this area.

It is a simple and well-known fact that most drugs are metabolized to variously different products via oxidation by hepatic cytochrome P-450 isoenzymes that demonstrate differing temporal oscillations. This fact is undoubtedly why a large number of studies published in the literature have failed to demonstrate temporal changes in elimination parameters such as plasma half-life and non-renal clearance. The *in vivo* characterization of selected cytochrome P-450 isoenzymes is and has been studied in both animals and man very adequately using drugs that are metabolized to one or two major metabolites in reactions catalyzed by one specific family of cytochrome P-450 isoenzymes. The only conditions for these types of studies are that the drug has to demonstrate a low hepatic extraction ratio. Examples of these types of compounds are phenylbutazone, tolbutamide, phenobarbital, antipyrine, phenytoin, and others. An approximate 40% decrease in the serum half-life of antipyrine in rats was observed when the drug was administered at 08:00 hour and an equivalent increase in the metabolic clearance of the drug when it was administered at 20:00 hour. Phenylbutazone and its major hydroxylated metabolite have been shown to demonstrate higher plasma levels at 20:00 hour and 01:00 hour than at 07:00 hour and 13:00 hour. With regard to the anti-inflammatory drug sundilac, higher plasma levels of the sulfone metabolite in humans have been observed when the drug was administered at 09:00 hour as opposed to 21:00 hour. Circadian oscillations in the pharmacokinetic profile for aminopyrene demonstrated a 50% increase in the half-life and a 20% decrease in the clearance value when administered at 20:00 hour when compared to an administration at 08:00 hour. Circadian changes or rhythms in various pharmacokinetic parameters have been reported in studies in humans administered carbamazepine, phenytoin, methotrexate, and caffeine. These and other reports in the literature demonstrate that the hepatic microsomal metabolism of drugs and xenobiotics in both animals and man are subject to diurnal variations in their pharmacokinetic profiles.

HEPATIC CONJUGATION REACTIONS AND DIURNAL VARIATIONS

In subsequent phase II reactions, these activated xenobiotic metabolites are conjugated with charged species such as glutathione (GSH), sulfate, glycine, or glucuronic acid. Sites on drugs where conjugation reactions occur include carboxyl ($-\text{COOH}$), hydroxyl ($-\text{OH}$), amino (NH_2), and sulfhydryl ($-\text{SH}$) groups. Products of conjugation reactions have increased molecular weight and tend to be less active than their substrates, unlike phase I reactions which often produce active metabolites. The addition

Table 6.3 Summary of Some of the Most Common Phase II Conjugation Reactions

Enzyme	Location	Mechanism of Action	Enzymatic Cofactor
Methyltransferase	Liver, kidney, lung, CNS	Methylation	S-adenosyl-L-methionine
Sulfotransferases	Liver, kidney, intestine	Sulphation	3'-phosphoadenosine-5'-phosphosulfate
• N-acetyltransferases • bile acid-CoA:amino acid N-acyltransferases	Liver, lung, spleen, gastric mucosa, RBCs, lymphocytes	Acetylation	Acetyl coenzyme A
UDP-glucuronosyltransferases	Liver, kidney, intestine, lung, skin, prostate, brain	Glucuronidation	UDP-glucuronic acid
Glutathione S-transferases	Liver, kidney	Glutathione conjugation	Glutathione
Acetyl co-enzyme As	Liver, kidney	Glycine conjugation	Glycine

of large anionic groups (such as GSH) detoxifies reactive electrophiles and produces more polar metabolites that cannot diffuse across membranes, and may, therefore, be actively transported [29,31,32,35–37,90,91,146,179,189,220,253,286,336,341].

These reactions are catalyzed by a large group of broad-specificity transferases, which in combination can metabolize almost any hydrophobic compound that contains nucleophilic or electrophilic groups. One of the most important classes of this group is that of the glutathione S-transferases (GSTs). Some of the most common phase II reactions are outlined in [Table 6.3](#).

After phase II reactions, the xenobiotic conjugates may be further metabolized via what is termed phase III modification. A common example is the processing of glutathione conjugates to acetylcysteine (mercapturic acid) conjugates. Here, the γ -glutamate and glycine residues in the glutathione molecule are removed by gamma-glutamyl transpeptidase and dipeptidases. In the final step, the cystine residue in the conjugate is acetylated.

Conjugates and their metabolites can be excreted from cells in phase III of their metabolism, with the anionic groups acting as affinity tags for a variety of membrane transporters of the multidrug resistance protein (MRP) family. These proteins are members of the family of ATP-binding cassette transporters and can catalyse the ATP-dependent transport of a huge variety of hydrophobic anions, and thus act to remove phase II products to the extracellular medium, where they may be further metabolised or excreted.

Phase II reactions are those that involve the conjugation of products generated from phase I and other xenobiotics containing functional groups such as hydroxyl, amino, carboxyl, epoxide, or halogens with endogenous metabolites. These endogenous metabolites include sugars, amino acids, glutathione, and sulfate. The conjugation products, with rare exceptions are more polar, less toxic, and more readily excreted than their parent compounds. Conjugation reactions usually involve high energy intermediates and have typically been classified into two

general types: type I (glycoside and sulfate formation) in which an activated conjugating agent combines with the substrate to yield a conjugated product; type II (amino acid conjugation) in which the substrate is activated and then combines with an amino acid to yield a conjugated product. The most important conjugation reactions are glycoside formation (especially glucuronidation), sulfate formation, glutathione conjugation, and mercapturic acid formation and acylation (especially amino acid conjugation). The literature is sparsely populated with reports on the circadian oscillations observed in non-oxidative reactions involved in drug metabolism. Indeed, only a couple of publications reporting on the circadian oscillation behavior in rat liver homogenate of the conjugation of glucuronic acid, sulfate, and acetate. In this work, the rates of glucuronidation and acetylation were found to be significantly elevated at 21:00 hour, when compared to the levels observed at 09:00 hour. However, sulfation was found to follow a reverse pattern with activity being twice as high at 09:00 hour when compared to 21:00 hour. Notably, these changes were found to be independent of protein concentration used in the assays. Interestingly, sulfotransferase activity was found to correlate with a four-fold decrease in the affinity of the substrate. The increased clearance of isoniazid was found to correlate well with a chronological pattern as regards the activity of N-acetyl transferase at 09:00 hour and 21:00 hour, with clearance being significantly higher at 21:00 hour. The circadian oscillatory pharmacokinetic behavior of hepatic sulfation and glucuronidation in both humans and rats may explain the diurnal variation in clearance of acetaminophen in humans and rats. The plasma half-life of acetaminophen in man was found to be approximately 20% longer at 06:00 hour than at 14:00 hour. Furthermore, the average ratio of glucuronide-acetaminophen conjugate to unaltered parent acetaminophen excreted in the first 3.5 hour urine sample varied from a value of 5:2 at 06:00 hour to a value of 7:8 at 14:00 hour. However, in the rat, it was observed that clearance and metabolism of acetaminophen determined as the extraction ratio were both greater in the evening than in the morning.

A significant source of sulfhydryl groups within the cell is the tripeptide compound glutathione. This compound in its reduced form has two main functions. Glutathione in its reduced form is a substrate for peroxidase redox system, which removes peroxides formed as a result of other enzymatically driven metabolic reactions. Additionally, glutathione in its reduced form and possibly most importantly forms adducts with electrophiles, free radicals or reactive intermediates of toxic drugs that are produced by the cytochrome P-450 monooxygenases. Succinctly put, the conjugation to glutathione is a process that protects vital cellular components from reactive oxygen species of xenobiotics. Prominent circadian oscillations in the hepatotoxicity of chloroform have been reported to be inversely related to the diurnal variation of the hepatic concentration of glutathione. The lowest levels of glutathione in the rat were found at 21:00 hour and the highest levels of glutathione were found at 09:00 hour, which correlates very well the maximal and minimal levels of hepatotoxicity. Similar results and correlations were found as regards the hepatotoxicity of 1,1-dichloroethylene, styrene, acetaminophen, and allyl alcohol.

INCIDENTAL PATHOLOGY OF THE CANINE GASTROINTESTINAL TRACT

The laboratory beagle is a nonrodent species that is frequently used in toxicology and safety assessment studies. These animals are purpose bred specifically for laboratory use and are unlike those beagles that are bred for use as pets or for hunting purposes. The lesions that are enumerated below are for the beagle bred for laboratory purposes and not for other beagles nor mongrel dogs that might be used in toxicology studies [3,24,38,46,47,63,69,104,115–117,125,147,149,159,163,168,186,188,198,241,270,272,274,282,290,294,310,335,362,369,386,418]. Furthermore, while these lesions or conditions may well occur in control groups, and even in test groups, they are generally not associated with test article toxicity. However they could be associated with test article toxicity as demonstrated by the presence of a dose-response pattern of incidence. Some organs that are worth commenting about follow:

The canine salivary gland can become inflamed. This sialoadenitis is generally mild in nature and of no consequence.

The canine tongue can uncommonly become inflamed (glossitis) in beagle dogs. Granulomatous inflammation can also occur, and is typically associated with housing dogs on sawdust bedding, where the tiny wooden fragments can become embedded in the tongue. Warts (papillomas) do occur and are benign epithelial neoplasms that can be found in young dogs and disappear as quickly as they appear.

The canine oral cavity can also develop papillomas on the lips, inside the cheeks as well as the tongue, palate, and pharynx.

The following lesions can be noted in the esophagus. Dilatation of the esophagus itself. Mild dilatation of the esophageal gland ducts. Hypertrophy or inflammation of the esophageal walls, arising as a result of mechanical irritation or reflux esophagitis.

The stomach in the canine has a number of findings as background lesions. Gastric glands can become dilated and such a finding is typically associated with the presence of spirillum-like bacteria. Gastric parietal cell atrophy can also be associated with the presence of spirillum-like bacteria. Lymphoid follicles or nodules are found to be commonly present in the lamina propria and are generally found to be present in the pyloric region of the stomach. Lymphoreticular hyperplasia in the stomach can be associated also with the presence of spirillum-like bacteria in the lumina of gastric glands and the intracellular canaliculi of parietal cells. Inflammation of the stomach can be acute or chronic in nature as well as granulomatous. Basophilic granules can occasionally be found in random locations in the middle fundic mucosa of young beagles and this is typically interpreted as simple mineralization.

The canine intestine is the site of a number of different lesions that are of little to no consequence. Simple congestion can be the result of agonal changes, inflammation, mechanical trauma, and even the process of digestion. Random in location mucoepithelial cysts can be occasionally observed. A diverticulum (e.g., Meckel's diverticulum) is a congenital abnormal sac or outpouching occurring at a weak point in the intestinal wall, typically the distal ileum and again is uncommonly found. This defect is best described as a pouch. Intestines can protrude through defects (typically congenital) in the abdominal wall forming a hernia. Not uncommonly

intestinal lymphoid hyperplasia can be observed. These are aggregates of lymphoid follicles and are typically found in the lamina propria. At necropsy these appear as little nodules. Inflammation of the large intestine can occasionally be found in the cecum and the colon. Inflammation of the granulomatous type can be observed in both the small and large intestines. The incidence of this type of inflammation is typically no more than a couple of percent. Intussusceptions have been noted to occur uncommonly in dogs. Intussusceptions occur when a segment of small or large intestine telescopes itself into a segment of contiguous intestine. Parvovirus is very rare situation as a result of vaccination, but can cause an acute enteritis where the intestinal wall as a result on the infection can become paper thin. Enteritis or inflammation of the small intestine is common and typically it is of the catarrhal type. Parasites are not common in laboratory-bred and -raised animals, but do occasionally occur. Various types of parasites that can be found are Metazoan parasites such as *Giardia* sp. and coccidia (*Isospora* sp.); Nematodes (roundworms or ascarids) such as *Ancylostomiasis* spp, *Toxocara canis*, *Toxascaris leonine* *Strongyloides stercoralia* and *Trichuris vulpis*. Cestodes or tapeworms are uncommon and the usual culprit is *Dipylidium caninum*. Keep in mind that many parasites can migrate through the body, causing additional lesions to their main locus.

The canine liver is host to a number of different types of lesions and quite commonly both gross and microscopic findings are noted. Subcapsular cysts do occur uncommonly in the beagle liver. Random in location foci of leukocytes are very commonly found in the young beagle dog liver and are typically found at an incidence of around 50%. Brown pigmentation can be seen in both hepatocytes and Kupffer cells. These brown pigmented granules are typically composed of either lipofuscin or hemosiderin or both mixed together. Bile pigment can also be present if biliary stasis is present in the liver. Acidophilic intracytoplasmic inclusion bodies can be found in the hepatocytes of the livers of beagle dogs. Even though these bodies stain positive with periodic acid-Schiff (PAS) reagents, they are not glycogen. Instead these inclusion bodies are proteinaceous material intermixed with lipid material. Acidophilic (hyaline) intranuclear inclusion bodies (rectangular, cubic, or rhomboid in shape) are frequently found in the nuclei of hepatocytes in the livers of beagle dogs. A focal subcapsular necrosis and lipidosis along with cytoplasmic vacuolization can quite commonly be found near the hilus of the liver at the base of insertion of the hepatic ligaments. This cell death results from tension on the hepatic capsule from the hepatic ligaments and the subsequent anoxia of adjacent hepatocytes and is frequently referred to as tension lipidosis. Random in location necrosis of hepatocytes is very commonly found in several different forms and is generally associated with degenerative and inflammatory lesions. Vacuolar degeneration of hepatocytes is not uncommon and considered to be a form of fatty change. Hepatic lipidosis, fatty change or fatty degeneration are not infrequently observed in the canine liver. Hepatic cytoplasmic vacuolization is commonly associated with the normal storage of glycogen. A large amount of cytoplasmic vacuolization can be expected to be seen after consumption of a meal. Alternatively, widespread cytoplasmic vacuolization can also be seen after a period of fasting (e.g., overnight). Bile duct hyperplasia in the canine liver is not very common and is typically found in association with portal

inflammation. Speaking of inflammation, the liver can be affected by inflammation in a variety of different ways. Mild portal inflammation with and without bile duct hyperplasia is quite common in the beagle liver. Inflammation of the bile duct is typically associated with necrosis or some other type of inflammation of the parenchyma of the liver and is relatively uncommon in occurrence. Very frequently hepatic veins can become inflamed such as an eosinophilic phlebitis or periphlebitis as a result of a hypersensitivity reaction to migrating parasites. However, the most common type of inflammation found in the beagle dog liver is of the granulomatous type. These lesions are composed of small focal collections of histiocytes, lymphocytes, rarely neutrophils, and a spattering of degenerate hepatocytes. The actual cause is unknown but at least some cases may well be associated with parasite migration (e.g., *Toxacara* spp).

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CHAPTER 7

Gastrointestinal Function and Toxicology in Minipigs

Maria R. Jones and Alain Stricker-Krongrad

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ANATOMY AND PHYSIOLOGY

The pig digestive system consists of primary digestive organs (mouth, esophagus, stomach, small, and large intestines), and accessory digestive organs (salivary glands, pancreas, liver, and gallbladder) (Figure 7.1). Pigs are both omnivores and monogastric, and their gastrointestinal systems are physiologically similar to humans. While

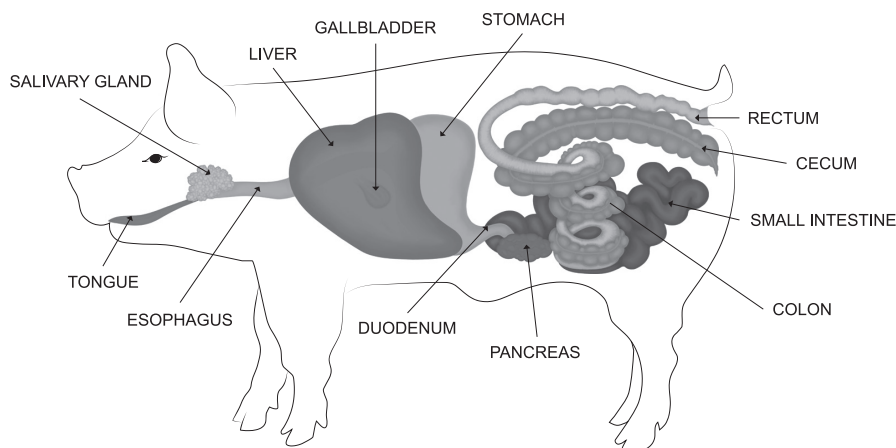


Figure 7.1 Illustration of minipig gastrointestinal organs.

domestic pigs grow to be quite large (>300 kg), minipigs are smaller (35–90 kg full-grown, dependent on breed) (Gad and Stricker-Krongrad 2016), making them easier to work with and more commonly used in biomedical research. While the primary function of the digestive system is to break down and absorb nutrients and liquids, an understanding of the anatomy and physiology of the pig's digestive system aids in species selection and interpretation in biomedical and pharmaceutical research.

The pig's oral mucosa consists of keratinized and non-keratinized epithelium and is more histologically similar to humans relative to other animal models (Herring et al. 2012, Thirion-Delalande et al. 2017). Adult minipigs teeth consist of three incisors, one canine, four premolar, and three molars (paired, mandibular, and maxillary). The minipig tongue occupies the majority of the oral cavity and is a muscular tissue covered with stratified squamous epithelium. While various shaped papillae cover the dorsal surface of the tongue, taste buds are located laterally and caudally within the circumvallate papillae and fungiform papillae (Herring et al. 2012). The minipig salivary glands consist of paired serous parotid glands, paired mixed (serous and mucous) submandibular gland, and paired (mostly mucous) sublingual glands. Both the parotid and submandibular glands of minipigs are similar in weight relative to human submandibular glands but have longer main ducts (Zhang et al. 2005, Herring et al. 2012). The serous fluid from parotid glands in minipigs consists of a combination of protein and electrolytes (Li et al. 2005, Gao et al. 2011, Zhu et al. 2016). Similar to humans, minipigs produce salivary amylase which aids in early carbohydrate digestion during mastication. The mucous production from the submandibular and sublingual glands aid in both lining the oral mucosa and food bolus for transit from the esophagus into the stomach.

The minipig pharynx contains a diverticulum and pharyngeal pouch, and thus proper training is necessary to avoid this when dosing by gavage in minipigs (Guthrie 1928, Bode et al. 2010). The esophagus is a muscular tube which extends from the pharynx to the stomach 12–14 mm in diameter in a collapsed state (McCrackin and

Swindle 2016). The musculature of the minipig esophagus is primarily smooth muscle, with some regions of striated muscle (Milano 2012, McCrackin and Swindle 2016).

The minipig stomach includes the esophageal, cardiac, fundic, and pyloric regions. The fundus contains the majority of the parietal and chief cells and is responsible for most of the acid production in the stomach, whereas the cardiac and pyloric regions contain mostly mucous cells (Kararli 1995, Van Ginneken 2012). The esophageal region of the pig stomach is non-glandular and is predisposed to ulceration (Doster 2000). Mucins secreted by the cardiac and pyloric glands line the stomach and prevent acid and pepsin from eroding the gastric mucosa (Allen and Garner 1980, Allen and Flemstrom 2005, Van Ginneken 2012). Notable anatomical differences in the pig stomach which is not present in humans include the diverticulum ventriculi and pyloric torus. The diverticulum ventriculi is present on the stomach near gastroesophageal junction, although its purpose is not fully understood (Hooper and Haelterman 1969, Kararli 1995, Van Ginneken 2012, Suenderhauf and Parrott 2013). The pylorus is made up of a semilunar muscular sphincter, and the pylorus torus is a protuberance occupying the lumen of the pylorus. Both the sphincter and pylorus torus have contractile ability, and function together to tightly close the stomach when chyme is not emptying into the small intestine (Bal and Ghoshal 1972).

Despite these differences, the minipig stomach has similar gastric secretions and pH to humans (Kararli 1995, Swindle and Smith 1998, DeSesso and Williams 2008). Parietal cells release both hydrochloric acid and intrinsic factor, which function to maintain an acidic pH in the stomach. Maintaining a low pH is crucial for initial denaturation of proteins and lipids, activation of proteolytic enzymes, and preventing microbial occupation and overgrowth in the stomach (Pohl et al. 2008). Chief cells produce pepsin and chymosin as zymogens, which become activated under acidic conditions (Van Ginneken 2012). While gastric lipases have been reported to be increased in weanling pigs (Jensen et al. 1997), the functional role of gastric lipases adult pigs remains unclear.

Gastric secretions are regulated by a combination of direct and indirect endocrine and neuronal signaling. The process of swallowing food stimulates a series of reflexes, which leads to parasympathetic release of acetylcholine in the stomach. Acetylcholine then directly stimulates parietal cells to release acid, reducing the pH of the stomach. Additionally, release of histamine and gastrin helps stimulate gastric secretion as well. As a negative feedback, the acidity of the stomach stimulates the release of somatostatin, which primarily inhibits gastric secretion and aids in lowering gastric pH as chyme is being released into the small intestine, which aids in absorption of nutrients. This also functions to keep pH low in the stomach between feeding, protecting the mucosa.

Peristaltic corporal-antral waves are initiated after ingestion of food, which aid in breakdown of solid foods. Liquids and fine solids have been shown to be rapidly emptied from the pig's stomach (Treacy et al. 1990, 1994, Milano 2012), although emptying is incomplete. Feeding regimen also appears to play a role in gastric emptying (Ruckenbusch and Bueno 1976), and it has been suggested that twice daily feeding in minipig may result in more predictable gastric emptying rates (Suenderhauf and Parrott 2013). Very little nutrient absorption occurs in the pig stomach (Burrin

and Stoll 2009); however, the gastric environment is crucial for modification of macronutrients and minerals (such as iron and calcium) to allow later absorption (Pohl et al. 2008).

The small intestine of adult minipigs ranges from 832 to 900 cm in length (Kurihara-Bergstrom et al. 1986, Suenderhauf and Parrott 2013) and consists of the duodenum, jejunum, and ileum. The duodenum is the first part of the small intestine. Digestive enzymes enter the duodenum from the liver through the ductus choledochus, while pancreatic digestive juices enter through a separate duct, the ductus pancreaticus. Chyme from the stomach mixes with pancreatic digestive enzymes and bile to continue digestion. Intestinal villi and microvilli are present in all areas of the small intestine, as well as intestinal crypts. Villi are the longest in the jejunum (Van Ginneken et al. 2002). Vascular density is increased in the jejunum in the pig relative to the ileum, making the jejunum poised for absorption. Pigs share some similarities in vascular features to humans along the intestines, but also show a fewer anastomoses relative to humans (von Trotha et al. 2015).

Brunner's glands are present in the minipig duodenum, and aid goblet cells (located along the length of the entire small intestine) in producing mucin and bicarbonate to increase intestinal pH, protect the intestinal mucosa, and aid in absorption of nutrients (Allen and Garner 1980, Allen and Flemstrom 2005, Van Ginneken 2012). The small intestine contains a large variety of enteroendocrine cells, and their function depends on location within the intestinal crypt. Deeper in the crypts the enteroendocrine cells have an endocrine function, and release hormones that influence a variety of intestinal activities (secretions, motility, etc.). Enteroendocrine cells located further from the crypts, towards the tips of the villus aid in absorbing nutrients. Peyer's patches are seen throughout the ileum of minipigs, with a long continuous band of Peyer's patches within the jejunum (Rothkotter and Pabst 1989). Motility within the small intestine of pigs is less variable than that of the stomach. While feeding regimen and meal size can affect intestinal motility (Ruckebusch and Bueno 1976), transit times of ingested solids and liquids are similar to that of humans (Suenderhauf and Parrott 2013).

Intestinal motility is influenced by fasting state, and during a fasting state the small intestine undergoes a migrating motor complex (MMC) in minipigs (Ruckebusch and Bueno 1976). Electrophysiologically, the MMC is characterized by regular periods of quiescence (Phase I), intermediate spiking (Phase II), and depolarization which leads to strong peristaltic contractions (Phase III). This allows for clearance of the intestines during periods of fasting. During feeding, the small intestine also undergoes a period of intermittent spiking, which allows for both segmental movement and peristalsis, aiding in the mixing and digestion of ingested contents and movement through the gastrointestinal system (Ruckebusch and Bueno 1976).

The majority of nutrient absorption occurs in the small intestine in the pig. Carbohydrates are digested into monosaccharides and primarily absorbed through sodium-dependent transporters and sodium independent transporters found on enterocytes in the duodenum and jejunum. Lipids are digested through a variety of lipases and bile salts to form micelles which can actively diffuse through the cell membrane of enterocytes. Lipid absorption can occur throughout the entire

small intestine but occurs primarily in the duodenum and jejunum. Amino acids, dipeptides, and tripeptides can be absorbed in the small intestine through a variety of sodium dependent transporters or peptide transport proteins, respectively (Van Ginneken 2012).

The large intestine consists the cecum, colon, and rectum. The cecum and colon are larger in minipigs than in humans (Suenderhauf and Parrott 2013). The colon is divided into the ascending, transverse, and descending colon. The ascending colon in minipigs is the largest portion of the colon and is unique in that it is spiraled (referred to as the *ansa spiralis*) (Van Ginneken 2012). The large intestine does not contain villi, but rather deep intestinal crypts. Columnar enterocytes with microvilli line the lumen of the colon, while crypts are made up of goblet cells and enteroendocrine cells. Peyer's patches can be found to continue from the small intestine to the cecum and proximal portion of the colon (Rothkotter and Pabst 1989, Van Ginneken 2012). Transit time through the large intestine of liquids and solids has been shown to be within a similar range to humans (Davis et al. 2001), although delayed or incomplete gastric emptying has the potential to make total GI transit times considerably longer in pigs (Hossain et al. 1990, Suenderhauf and Parrott 2013). In contrast to the small intestine, nutrient absorption in the cecum and large intestine is reduced relative to the small intestine, with short-chain fatty acids, salt and fluid reabsorption from gastrointestinal secretions being primarily what is absorbed in this region.

The liver has six lobes separated by fibrous tissues, and the gallbladder is a sac located under the right central lobe. Minipig liver lobules have a portal triad (central artery, vein, and bile duct). Bile produced by pigs has a similar composition (Alvaro et al. 1986, Preube and Skaanild 2012) but is less concentrated than human bile (Nakayama 1969). While bile production is important to gastrointestinal physiology, the liver of minipigs shares many of the cytochromes seen in humans (Soucek et al. 2001, Skaanild and Friis 2002), making the pig an excellent model for drug absorption, metabolism, and toxicology.

The pancreas of the minipig consists of a head, a body which splits into an anterior and posterior segment, and tail (Tsuchitani et al. 2016). The pancreas is large, and extends anteriorly towards the spleen, and retroperitoneally towards the right kidney, with both lobes encircling the portal vein (Ferrer et al. 2008, Swindle et al. 2012, Tsuchitani et al. 2016). The minipig pancreas is functionally similar to humans in both endocrine and exocrine function, which makes it an a model used extensively for diabetes (Larsen and Rollin 2004, Stricker-Krongrad et al. 2016a), pancreatic development (Jensen et al. 1997, Rantzer et al. 1997), and function (Houe et al. 1997, Vinter-Jensen et al. 1997, Mosseler et al. 2015).

Key differences in anatomy and physiology are found during development of the minipig GI system. In particular, the stomach and small intestine go through morphological and physiological changes from late in gestation, to post-weaning. The stomach is less acidic during gestation and early postnatal days, and gradually lowers within the first month (Van Peer et al. 2016). The small intestine undergoes morphological changes in maturation of villi and intestinal crypts. Pancreatic secretions have been shown to increase in the week following weaning in piglets (Rantzer et al. 1997), although total pancreatic activity of trypsin, chymosin, and amylase

in the pancreas appears to increase 2–4 weeks post weaning (Jensen et al. 1997). *In vitro* CYP450 of the liver is low in early postnatal piglets, but increases markedly by Day 28, and continues to increase into adulthood (Van Peer et al. 2017). Additionally, CYP3A is expressed at lower levels in both the small intestine and liver and increases as piglets age in a similar pattern as piglets age (Van Peer et al. 2014).

MINIPIGS IN GASTROINTESTINAL SURGERY AND EFFICACY MODELS

Minipigs are frequently used for gastrointestinal surgical procedures, implants, repair models, and grafts. Surgical procedures designed to aid in weight reduction have been performed in minipigs. A Roux-en-Y Gastric Bypass was performed in Yucatan, Hanford, and “Other Breed” minipigs. This procedure resulted in a many postoperative complications, including a total of one intraoperative, and five post-operative deaths (primarily via humane euthanasia due to clinical signs). Food consumption in surviving animals 1 month post-surgery was variable among individuals and breeds. Hanford minipigs continued eating all of their food, and overall gained weight, although less weight than expected for food consumption. Yucatan and other breed minipigs demonstrated overall weight loss, with much higher variability in food consumption (Flum et al. 2007). Endoscopically introduced gastric balloons have been examined in Göttingen minipigs and were shown to affect gastric volume and rate of emptying. Although this model appears to induce minimal overall complications, there was no observed weight loss observed during this study (Layec et al. 2010).

Additional studies have used minipigs to measure overall effectiveness and side-effects of implants intended to prevent gastric reflux. One study using Yucatan minipigs showed implantation of such material caused inflammatory reactions acutely, and eventually lead to implants becoming surrounded by fibrous capsules (Mason et al. 2002). Another study assessed the effect of a different type of implant which also showed inflammation and surrounding of polymer implanted spheres by fibrous tissues (Fornari et al. 2009). Minipigs are also frequently used in hernia repair models to assess overall safety of devices (i.e., surgical mesh) to enhance hernia healing and prevent complications from abdominal incisions (Jenkins et al. 2010, 2011, Petter-Puchner et al. 2010, Melman et al. 2011, Monteiro et al. 2013, Cavallo et al. 2015), and to assess safety and efficacy of pharmaceutical compounds (such as pain relievers) in conjunction with hernia models (Gadsden and Long 2016).

Minipig salivary glands are also used in surgical/efficacy research. While many studies have been done to assess the effects of radiation on salivary glands (described later in this chapter), additional studies have been performed to assess the feasibility of salivary gland tissue grafts (Ge et al. 2006), or treatment of excessive salivation by cryoablation of salivary tissue (Buethe et al. 2016). Cryoablation of parotid glands showed lymph nodes within the tissue and fibrous tissue formation, but no apparent abnormalities were detected in a CT scan (Buethe et al. 2016). In the tissue grafting

study, there was a dose-dependent prevention of tissue rejection when mandibular glands were grafted in minipigs, although the highest dose of immunosuppressants still only resulted in 2 of 6 salivary glands being viable after 100 days. Three glands were rejected, and one animal died early on study (Ge et al. 2006). Although many immunological processes within the minipig remain uncharacterized, its size and anatomic similarity to humans make it a useful for transplantation models.

DRUG ABSORPTION IN MINIPIGS

Orally dosed pharmaceuticals make up the highest percentage of drug development and are favorable for drug administration. The minipig is useful for oral administration of pharmaceuticals due to similar regional pH of the GI tract and intestinal transit times (Davis et al. 2001). Additionally, the size of minipigs allows for large volumes of blood to be collected, making them ideal for pharmacokinetic (PK) assessment of compounds. The most commonly used breeds in Toxicology and PK studies are the Sinclair, Hanford, Yucatan, and Göttingen minipigs (Figure 7.2) (Ganderup et al. 2012, Swindle et al. 2012, Stricker-Krongrad et al. 2016b, Helke et al. 2016b).

It has been shown there are breed specific differences in pharmacokinetic profiles of intravenously and orally administered drugs. Recently, the pharmacokinetic profile of Metformin and verapamil was assessed in all four breeds of commonly used minipigs. Metformin is an orally administered drug for treatment of hyperglycemia in type II diabetes. Metformin does not undergo first-pass metabolism

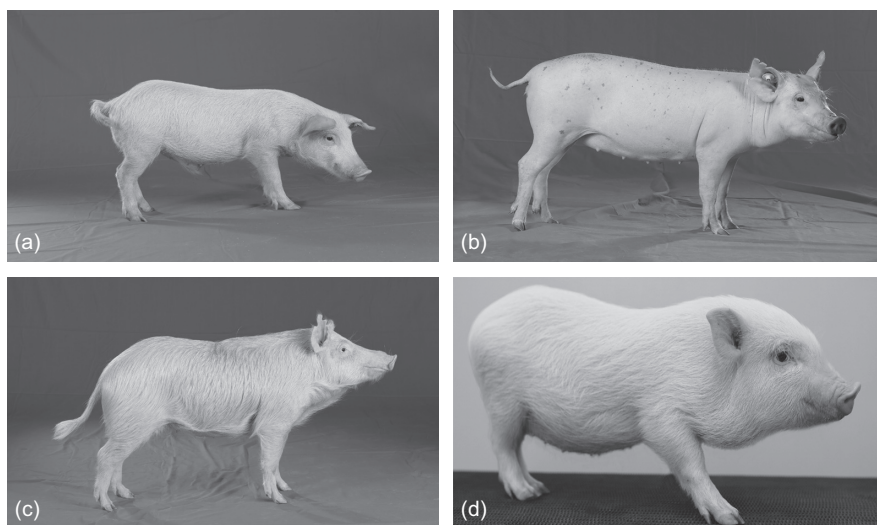


Figure 7.2 Representative images for (a) Hanford, (b) Yucatan, (c) Sinclair, and (d) Göttingen minipigs.

through the liver, but rather is rapidly renally cleared and excreted (Graham et al. 2011). Verapamil, a calcium channel blocker used to treat hypertension, undergoes extensive metabolism and forms several inactive metabolites, as well as the active metabolite nor-verapamil (Tracy et al. 1999, Bhatti and Foster 1997). Additionally, it is a chiral drug, with species specific differences in preferential metabolism of R- and S-verapamil and nor-verapamil (Patel et al. 2017).

Sinclair, Yucatan, and Hanford breeds had similar rates of clearance of intravenously administered metformin, but Göttingen minipigs cleared IV administered metformin at nearly twice the rate as the other breeds (Figure 7.3a). In contrast, oral administration of metformin resulted in similar systemic exposures over a 2-day period among all four breeds (Figure 7.3b). When considering an increased glomerular filtration rate in minipigs relative to humans, clearance of metformin in Göttingen minipigs is most similar to human clearance when administered intravenously. Interestingly, the oral bioavailability of metformin was more similar in Sinclair, Yucatan, and Hanford minipigs when compared to humans. Oral bioavailability of metformin was considerably higher in Göttingen minipigs than any of the other breeds or humans, but that number is likely skewed due to the shorter time interval in the IV profile vs. oral profile (8 vs. 48 hour, respectively) (Patel et al. 2017).

In contrast with the pharmacokinetics profile of metformin, Sinclair minipigs cleared verapamil nearly two times slower than Yucatan, Hanford, or Göttingen

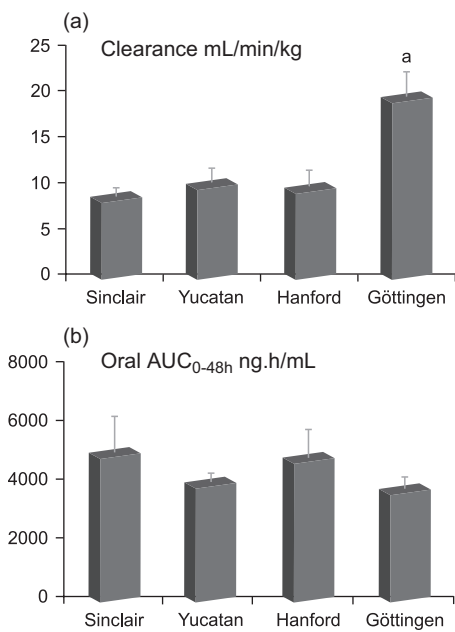


Figure 7.3 (a) Mean intravenous clearance (mL/min/kg) or (b) Oral AUC_{0-48h} ng.h/mL of metformin in commonly used breeds of minipigs. (Data from Patel, N.J. et al., *Int. J. Pharmacokinet.*, 2, 81–91, 2017.)

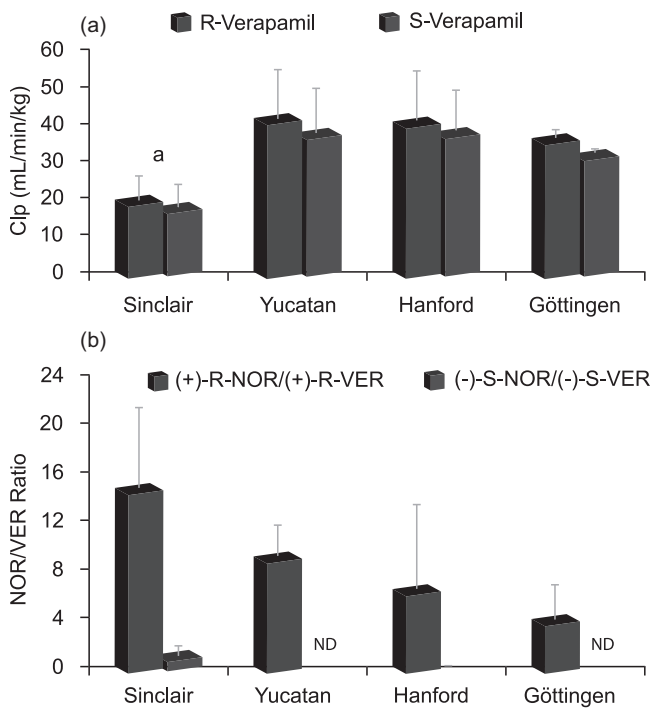


Figure 7.4 (a) Mean intravenous clearance (mL/min/kg) or (b) NOR/VER ratio of verapamil or nor-verapamil in commonly used breeds of minipigs. (Data from Patel, N.J. et al., *Int. J. Pharmacokinet.*, 2, 81–91, 2017.)

minipigs when administered intravenously (Figure 7.4). Additionally, both R-verapamil and S-verapamil were present in the plasma, and there was no preferential clearance of either R-verapamil or S-verapamil in any breed, whereas humans demonstrate a preferential clearance of S-verapamil compared to R-verapamil (Vogelgesang et al. 1984, Patel et al. 2017).

The differences found in minipig stomach play a major role in selecting formulations for assessment. Pig stomach pH values reported are variable and appear to be dependent on fasted state (Oberle and Das 1994), region of the stomach (Kararli 1995), and method of data collection (Moeller et al. 1974, Oberle and Das 1994, Kararli 1995), but nevertheless has been shown to have a similar pH range relative to humans (Moeller et al. 1974, Kararli 1995, Swindle and Smith 1998), which is beneficial for assessment of pharmaceuticals. However, the differences in stomach anatomy and motility should be considered when selecting dosing formulations. Gastric emptying in pigs is biphasic, where a portion of the stomach is emptied within 15 minutes following a meal, and larger portion is emptied approximately one hour post feeding (Preube and Skaanild 2012). While gastric emptying of liquids and finely ground solids occurs within quickly (Treacy et al. 1990, Van Ginneken 2012), gastric emptying appears to be incomplete in pigs

(Milano 2012), which has significant implications in interpreting absorption of a pharmaceutical compounds. Larger solids, such as capsules, can take a few hours to several days to exit the stomach in pigs (Hossain et al. 1990, Aoyagi et al. 1992, Oberle and Das 1994, Davis et al. 2001). This is likely due to the pylorus torus preventing passage of larger solids in minipigs. Thus, administering liquid formulations dosed by gavage is ideal when assessing absorption of pharmaceuticals in minipigs.

A concern in drug absorption and bioavailability for oral compounds is first-pass metabolism through the liver, which can substantially reduce bioavailability. In order to enhance bioavailability, formulations are being developed for buccal administration. This allows for direct systemic absorption into the bloodstream through the oral mucosa. In fact, *in vitro* and *ex vivo* studies have used minipig and pig oral mucosa to assess permeability (Liu et al. 2002, Adhikari et al. 2010, Kumria et al. 2016). Furthermore, *in vivo* evidence suggests buccal administration and formulations have the potential to greatly enhance bioavailability in minipigs, where buccal administration of diazepam increased bioavailability up to 76%, whereas standard oral administration of similar dose levels resulted in bioavailability being 30% (Meng-Lund et al. 2016). Taken together, this suggests buccal formulations could be a useful to increase system exposure and avoid first-pass metabolism by the liver.

TOXICOLOGY

Minipigs have been gaining popularity in research over dogs and non-human primates (NHP), particularly in Canada and the European Union, where swine use outnumbers that of dog or NHP (Ganderup et al. 2012). Since pigs are generally considered livestock rather than a companion animal, less opposition is generally found in their use in research. Minipigs are well-suited for toxicology an anatomical and physiological perspective. However, while there are up to 20 minipig breeds described in the literature (Ganderup et al. 2012, Köhn 2012), only four breeds are generally used in toxicology studies due to available background data and breed specifications. These include Sinclair, Hanford, Yucatan, and Göttingen minipigs. They are sexually mature between 3 and 5 months of age (Ganderup et al. 2012, Swindle et al. 2012), allowing younger animals to be used. Minipigs are frequently used for dermal toxicity studies (Stricker-Krongrad et al. 2017), as their skin is very similar to human skin; however, they are also used for oral toxicity studies as well (Ganderup et al. 2012). A thorough review of marketed products that underwent some part of their pre-clinical safety/efficacy studies in minipigs showed minipig results had a high predictive value of potential adverse effects of marketed products (Ganderup et al. 2012). Incidental findings occur in the minipig, and it is useful to be aware of the incidental findings that occur in the minipig GI system. Here we present tabulated incidental background histopathological changes in all four breeds of minipigs (Table 7.1) and discuss additional incidental findings in minipig breeds described in the literature.

Table 7.1 Most Common Incidentally Occurring Microscopic Findings in Control Minipigs Gastrointestinal System

Organs/Tissues	Sinclair		Hanford		Yucatan		Göttingen	
	Male (N = 11)	Female (N = 11)	Male (N = 60)	Female (N = 59)	Male (N = 18)	Female (N = 21)	Male (N = 143)	Female (N = 143)
Tongue								
Inflammation, focal, minimal to slight	—	—	1	—	—	—	5	8
Ulceration, focal, slight	—	—	—	—	—	—	1	—
Salivary Glands								
Mononuclear infiltrate, minimal	—	—	2	2	1	1	6	3
Lymphohistiocytic inflammation, multifocal, minimal to mild	1	—	—	1	—	—	—	—
Mineralization, glandular, focal, minimal	—	—	—	—	—	—	—	1
Esophagus								
Erosion, minimal	—	—	—	—	—	1	—	—
Hyperkeratosis, slight to mild	—	—	1	—	—	—	—	—
Increased submucosal lymphoid nodules, slight to moderate	—	—	18	8	—	—	—	—
Infiltration, lymphohistiocytic, multifocal, moderate	—	—	—	1	—	—	—	—
Mononuclear infiltrates, minimal	—	—	—	—	1	—	2	2
Inflammation, neutrophilic, mucosa, focal, minimal	—	—	—	1	—	—	—	—
Inflammation, lymphohistiocytic, multifocal, minimal	—	1	—	—	—	—	—	—
Inflammation, acute, mild	—	—	—	1	—	—	—	—

(Continued)

Table 7.1 (Continued) Most Common Incidentally Occurring Microscopic Findings in Control Minipigs Gastrointestinal System

Organs/Tissues	Sinclair (N = 11)		Hanford (N = 60)		Yucatan (N = 18)		Göttingen (N = 143)	
	Male (N = 11)	Female (N = 11)	Male (N = 60)	Female (N = 59)	Male (N = 18)	Female (N = 21)	Male (N = 143)	Female (N = 143)
Stomach								
Infiltration, lymphocytic, muscularis, multifocal, minimal	—	—	2	3	—	—	—	—
Mononuclear infiltrates, minimal	—	—	—	—	1	1	—	—
Inflammation, neutrophilic, esophageal region, multifocal, mild	—	—	—	1	—	—	—	—
Inflammation, lymphohistiocytic, muscularis, multifocal	2	—	—	—	—	—	—	—
Inflammation, acute, minimal	—	—	—	—	—	1	—	—
Inflammation, mucosal, focal, minimal	—	—	—	—	—	—	4	3
Hemorrhage, fundus, multifocal, mild	—	—	—	1	—	—	—	—
Erosion	1	—	—	—	—	1	1	2
Hyperkeratosis, minimal	—	—	—	—	—	1	—	—
Increased mucosal/submucosal lymphoid nodules, slight to moderate	—	—	22	21	—	—	—	—
Small Intestine								
Jejunal mineralization, multifocal, minimal	—	—	—	1	—	—	—	—
Ileal inflammation, acute, minimal	—	—	1	—	—	—	—	—
Inflammation, focal to diffuse, minimal to marked	—	—	—	—	—	—	5	2
Peritonitis, focal, minimal to slight	—	—	—	—	—	—	—	2

(Continued)

Table 7.1 (Continued) Most Common Incidentally Occurring Microscopic Findings in Control Minipigs Gastrointestinal System

Organs/Tissues	Sinclair		Hanford		Yucatan		Göttingen	
	Male (N = 11)	Female (N = 11)	Male (N = 60)	Female (N = 59)	Male (N = 18)	Female (N = 21)	Male (N = 143)	Female (N = 143)
Eosinophilic infiltrates, ileum, minimal	—	—	—	—	—	1	—	—
Mononuclear infiltrates, ileum, slight	—	1	—	—	—	—	—	—
Large Intestine								
Hemorrhage, multifocal, minimal	—	1	—	—	—	—	—	—
Herniation, mucosa into submucosal lymphoid nodule, minimal	—	—	—	1	—	—	—	—
Inflammation, focal to diffuse, minimal to moderate	—	—	—	—	—	—	5	1
Arteritis, chronic, focal, minimal	—	—	—	—	—	—	—	1
Liver								
Inflammation, lymphohistiocytic, multifocal, minimal	1	2	2	2	—	—	—	—
Inflammation, chronic, lobular, minimal	—	—	15	11	—	—	—	—
Inflammation, chronic, minimal	—	—	9	9	—	—	—	—
Inflammation, multifocal, random, subacute, minimal	—	—	4	1	—	—	—	—
Inflammation, chronic, portal, minimal	—	—	—	3	—	—	—	—
Mononuclear infiltrates, minimal	—	—	—	—	4	6	7	12
Vacuolation, hepatocyte, diffuse, minimal to mild	—	—	—	2	—	—	—	—
Hemorrhage, acute, peribiliary, slight to mild	—	—	—	1	—	—	—	—
Necrosis, focal, minimal	—	—	—	1	—	—	—	—

(Continued)

Table 7.1 (Continued) Most Common Incidentally Occurring Microscopic Findings in Control Minipigs Gastrointestinal System

Organs/Tissues	Sinclair		Hanford		Yucatan		Göttingen	
	Male (N = 11)	Female (N = 11)	Male (N = 60)	Female (N = 59)	Male (N = 18)	Female (N = 21)	Male (N = 143)	Female (N = 143)
Necrosis, parenchymal, focal, minimal to slight	—	—	—	—	—	—	—	2
Single cell necrosis, minimal	—	—	—	—	—	—	—	1
Fibrosis, interlobular, minimal	—	—	—	—	—	—	1	—
Abscess, present	—	—	—	1	—	—	—	—
Gallbladder								
Increased submucosal lymphoid nodules, slight to mild	—	—	1	—	—	—	—	—
Cholecystitis, diffuse, marked	—	—	—	—	—	—	1	—
Pancreas								
Mononuclear cells, interstitial, focal, minimal	—	—	—	—	—	—	1	1
Hemorrhage, interstitial, minimal	—	—	—	—	—	—	—	2
Arteritis/periarteritis, necrotising, minimal	—	—	—	—	—	—	—	1

Background Findings in Minipig Breeds

Tongue

Slight focal inflammation is seen in both Hanford and Göttingen minipigs but is quite rare. Most incidental findings can be attributed to dose route (oral gavage) or simple mechanical damage (biting the tongue). This has been demonstrated repeatedly in Göttingen minipigs, which also show mild myositis of striated muscle (Gad and Stricker-Krongrad 2016), mild focal necrosis (Jeppesen and Skydsgaard 2015, Helke et al. 2016a, 2016b), erosion, and/or ulcerations (Jeppesen and Skydsgaard 2015). Similar incidental findings (inflammatory cell infiltrate and hemorrhage) have also been reported in Yucatan minipigs (Helke et al. 2016a). Additionally, ectopic chondrocytes within the tongue occur in Göttingen minipigs, which are not believed to be due to any inflammatory response (McInnes and McKeag 2016).

Salivary Glands

Mononuclear cell infiltrate at a minimal level occurs occasionally in Hanford, Yucatan, and Göttingen minipigs. Minimal focal mineralization is occasionally seen in Göttingen minipigs. Our findings concur previously published findings (Jeppesen and Skydsgaard 2015, Gad and Stricker-Krongrad 2016, Helke et al. 2016a, 2016b, Stricker-Krongrad et al. 2016b). In addition, Göttingen minipigs have been reported to have submandibular gland edema occasionally at necropsy (Gad and Stricker-Krongrad 2016), and histologically glands can incidentally show mineralization and sialolith (Helke et al. 2016a, 2016b). Metaplasia of the cuboidal epithelium can also be found in Göttingen minipigs (McInnes and McKeag 2016). Yucatan minipigs have also been reported to have cellular vacuolization and mineralization within salivary glands (Helke et al. 2016a, 2016b).

Esophagus

Hanfords have increased slight to moderate submucosal lymphoid nodules in the esophagus, which are not reported in other breeds, as well as rare hyperkeratosis. Immune cell infiltration can be seen in all four breeds of minipigs used in toxicology studies. Our data showed ulceration as a background finding in Yucatan minipigs, but erosion and ulceration have been described in Yucatan, and Göttingen minipigs previously (Helke et al. 2016a, 2016b). Incidental hemorrhage around the esophagus associated with blood collection has been described in Göttingen minipigs (Jeppesen and Skydsgaard 2015).

Stomach

Hanfords have increased slight to moderate submucosal lymphoid nodules in the stomach, which are not reported in other breeds. Minimal to mild inflammation is an incidental finding common to all four breeds of minipigs. Macroscopic findings

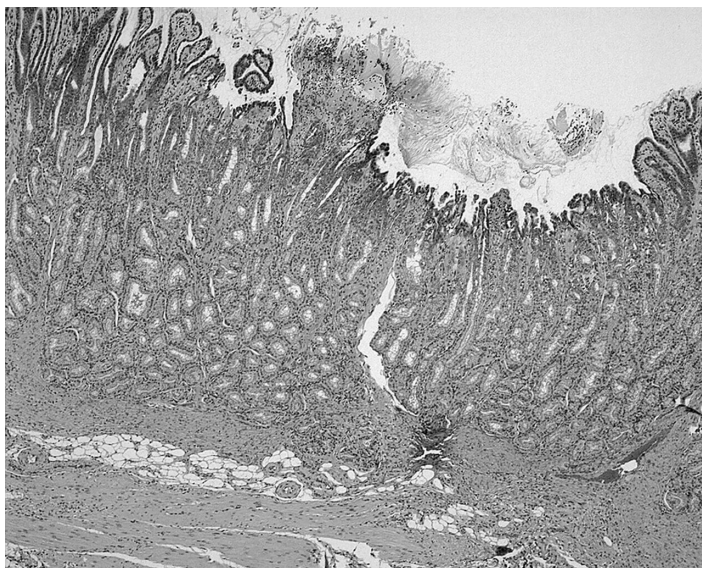


Figure 7.5 Acute erosion of glandular stomach in Götting minipig. (Reprinted from Gad, S.C. and Stricker-Krongrad, A., The minipig, In *Animal Models in Toxicology*, edited by S.C. Gad, pp. 763–808, CRC Press, Boca Raton, FL, 2016.)

include adhesions of the stomach in Hanford, and discoloration in Hanford and Yucatan minipigs (Stricker-Krongrad et al. 2016b). Erosion of the stomach is seen in Sinclair, Yucatan, and Göttingen minipigs in our data set, but erosion the stomach has been reported previously in Hanford minipigs as well (Helke et al. 2016a, 2016b). Focal acute erosion in the glandular portion of the stomach of Göttingen minipig is shown in [Figure 7.5](#).

Small Intestine

Inflammation and peritonitis is seen in the small intestine of Göttingen minipigs. Mononuclear cell infiltrates can be found in rare instances in Sinclair minipigs, while eosinophilic infiltrates are found on rare occasions in Yucatan minipigs. Mineralization and inflammation of the small intestine was seen in Hanford minipigs, rarely. Additional incidental findings in the Göttingen minipig that have been reported include erosion, villous atrophy, as well as focal arteritis, phlebitis, and fibrosis (Jeppesen and Skydsgaard 2015, Helke et al. 2016a, 2016b).

Large Intestine

Minimal to moderate inflammation as well as focal arteritis can be observed in the large intestine Göttingen minipigs. Additionally, focal peritonitis and submucosal edema have been reported in Göttingen minipigs previously (Jeppesen and

Skydsgaard 2015). Rare instances of hemorrhage have been shown in Sinclair minipigs, and rare herniation was seen in the large intestine of Hanford minipig.

Liver

Inflammation of liver appears to be a more common incidental finding in Hanford minipigs, while Yucatan and Göttingen show mononuclear infiltrates. Necrosis and fibrosis are rare incidental findings in the Göttingen minipig. At necropsy, macroscopic adhesions and abscesses can be seen in Hanford minipigs (Stricker-Krongrad et al. 2016b). We observed a single instance of hepatocyte vacuolation in a Hanford minipig, but this has also been reported in Yucatan and Göttingen minipigs (Helke et al. 2016a, 2016b). One study described incidental histopathology findings in young Göttingen minipigs, including degeneration, necrosis, hemorrhage, and congestion, that were likely caused by firm handling during dosing (Ramot et al. 2016).

Gallbladder

Hanfords have submucosal lymphoid nodules in the gallbladder rarely. Göttingens have been showed to have necrotizing cholecystitis ([Figure 7.6](#)), hemorrhage, inflammation and inflammatory cell infiltrate, and aplasia (Helke et al. 2016a, 2016b, Stricker-Krongrad et al. 2016b).



Figure 7.6 Chronic necrotizing cholecystitis in the minipig gallbladder. (From Gad, S.C. and Stricker-Krongrad, A., The minipig, In *Animal Models in Toxicology*, edited by S.C. Gad, pp. 763–808, CRC Press, Boca Raton, FL, 2016.)

Pancreas

Across all breeds of minipig, incidental findings in the pancreas are rare. Göttingen minipigs have been shown to have interstitial mononuclear cell infiltration, minimal hemorrhage, and necrotizing arteritis, which is consistent with previous reports (Helke et al. 2016a, 2016b, Jeppesen and Skydsgaard 2015).

Minipig Gastrointestinal Toxicity

Chemotherapeutic agents are successful in targeting harmful cancer cells, often causing associated damage to healthy tissues as well. One anti-cancer agent (name not provided) was a small molecule intended to have immunosuppressant activities was tested in oral gavage in multiple species. Doses were tolerated better in minipigs than dogs, but resulted in coccidial infestation, which lead to inflammation, and ulceration, and villous atrophy of the intestines. These lesions were considered secondary in nature due to the immunosuppressant activities of the drug (Mahl et al. 2016). Doxorubicin, while primarily a cardiac toxin, has been shown to cause villous atrophy of the small intestine of minipigs (Manno et al. 2016).

Vehicle-Induced Toxicity in Oral Compounds

Vehicles themselves can cause toxic effects, and those should be considered when selecting formulations. One example used in minipigs is Miglyol 812. When dosed with 2 mL/kg/day animals showed gastrointestinal symptoms such as loss of appetite, dry/dark feces, or few feces. Abdominal spasms and vomiting occurred during preliminary studies when dosing minipigs at 10 mL/kg. Histopathological changes to the GI system were considered incidental and not associated with Miglyol 812. However, the primary lesions described were to the lungs, and could be due to regurgitation and subsequent aspiration of the viscous material dosed by oral gavage (Le Bars et al. 2015). Understanding potential adverse reactions of a vehicle is crucial in making toxicology determinations. Short-term oral administration of low levels (0.5% in distilled water) of carboxymethylcellulose or hydroxypropyl methylcellulose did not show any adverse reactions when orally dosed in minipigs (at 8 mL/kg and 5 mL/kg, respectively). However, 90-day daily administration of Transcutol has been shown to be toxic when orally dosed in minipigs, causing liver and kidney damage, with the NOAEL being 167 mg/kg/day. Mixtures of vehicles with low levels of transcutol for short-term oral dosing have shown mild transient diarrhea after initial dose, but a different formulation showed no adverse reactions. A summary of safety assessments of orally dosed vehicles is presented in [Table 7.2](#) (Gad et al. 2016).

Radiation-Induced Damage to the Gastrointestinal System in Minipigs

The nature of gastrointestinal tissue makes it sensitive to irradiation. Minipigs have recently been used as models of toxic effects of radiation. Studies have been done looking at total body irradiation, and irradiation to specific organs. The effects

Table 7.2 Tolerability of Orally Administered Vehicles in Minipigs

Vehicle(s)	Duration	Dose	Adverse Reactions/ Toxicity	Notes
Carboxymethylcellulose	14 days	8 mL/kg SD	None	0.5% CMC in water; GLP; age 3 months; 1 male/1 female
	28 days	8 mL/kg QD	None	0.5% CMC in water; GLP; age 3–4 months; 4 males/4 females
Hydroxypropyl Methylcellulose (Gavage)	7 days	5 mL/kg QD	None	0.5% in distilled water; age 6.5–11.5 months; male/female
Phosphate-Buffered Saline	7 days	5 mL/kg BID	Well-tolerated	GLP; age 4 days; 4 males/4 females
	28 days	5 mL/kg BID	Well-tolerated	GLP; pH 6.0; age 4–5 days; 10 males/10 females
Transcutol	90 days	0, 167, 500, 1,500 mg/ kg/day	NOAEL = 167 mg/kg/d	Uremia, death at 1,500 mg/kg/d; high dose reduced to 1,000 mg/kg/d after 21 days; histopath in doses >500 mg/kg/d includes hydropic degeneration of liver and proximal kidney tubules; at >1,000 mg/kg/d increased relative kidney weight and decreased RBC (males)
	4 days	5 mL/kg QD	None	Non-GLP; age 4–6 months; 3 females

(Continued)

Table 7.2 (Continued) Tolerability of Orally Administered Vehicles in Minipigs

Vehicle(s)	Duration	Dose	Adverse Reactions/ Toxicity	Notes
Gelucire 44/14/PEG 400/NMP/ Transcutol HP (50/30/10/10)	3 days	5 mL/kg QD	Mild transient diarrhea 24–30 hours after first dose	Non-GLP; age 7–8 months; 3 females
HPMC acetate succinate (90 mg/ mL)/methylcellulose (0.5%)	14 days	6 mL/kg EOD	None	GLP; age 3–5 months; 5 males/5 females
Labrasol/Kolliphor HS15/ Transcutol HP (60/30/10) (Gavage)	4 weeks	3.75 mL/kg Once weekly	None	Age 6–7 months; male/female

Source: Gad, S.C. et al., *Int. J. Toxicol.*, 35, 95–178, 2016.

of radiation on the GI system are marked, and of interest in instances of chemotherapy requiring radiation, or in instances of radiation exposure. Early studies in minipigs demonstrated a dose-dependent effect of total body irradiation and mortality. Early mortality in the high doses of irradiation were associated with severe lesions to the GI tract, while later mortality in the lower doses of irradiation were associated with bone marrow deficiencies (Jones et al. 1972). These findings have since been replicable in Göttingen minipigs, where high levels of radiation can induce Gastrointestinal Acute Radiation Syndrome, which demonstrates vomiting, diarrhea, and reduced body weights (Shim et al. 2014), as well as loss of intestinal crypts and villi (Elliott et al. 2014). Total body irradiation results in significant alterations to mitochondrial structure and function within the GI system (Wang et al. 2015). Recently, plasma citrulline has been used as a biomarker for acute radiation syndrome and appears to be reduced in minipigs after radiation induced GI injury (Jones et al. 2014, Bujold et al. 2016).

In addition to inducing GI injuries consistent with total body irradiation, direct radiation to tissue can induce damage that is not necessarily lethal. Irradiation of salivary glands of minipigs has been used to model xerostomia, caused by head/neck radiation for therapeutic purposes. Irradiated salivary glands in Hanford minipigs at therapeutic doses (7000 cGy total over 35 separate treatments) are significantly smaller, and both parotid and submandibular glands show fibrosis, acinar atrophy, and parenchymal loss as well as ductal dilation and proliferation. As a result, salivation was significantly reduced (Radfar and Sirois 2003). Similar studies showed alterations in salivary amylase and electrolytes (Li et al. 2005), damage to microvasculature, and altered perfusion of salivary glands (Xu et al. 2010). Minipigs as a robust and reproducible model of salivary gland defects have been used to assess therapies to target irradiation induced salivary dysfunction, including virally delivered (Gao et al. 2011) and non-virally delivered (Wang et al. 2015) gene therapies for aquaporin-1, immunosuppressant drugs (Zhu et al. 2016), and stem cell therapies (Chen et al. 2014). These have all shown some success in restoring salivary function in irradiated minipigs. Additionally, minipigs have been used to model irradiation induced oral mucositis—showing ulcerations, erosion, and epithelial changes in the oral mucosa (Hu et al. 2017).

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CHAPTER 8

Absorption of Macromolecules by Mammalian Intestinal Epithelium

Shayne C. Gad

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Primary human oral absorption of xenobiotics is, of course, dependent on a wide range of mechanisms which are dictated by both the structures in question and the selectivity and functionality of all the aspects of the bodies GI physiology and biochemistry, as well as the influence of its microbiome.

Basic patterns of metabolism are a good place to start. Commonly used for describing the chemical/metabolic modification of xenobiotics is the three-phase model: Phase I (mainly oxidative functional group changes, in the majority by cytochrome P450s), Phase II (conjugating enzymes that usually add large hydrophilic molecules to the molecules in Phase I metabolite), and finally Phase III (elimination systems that facilitate the removal of the metabolites from cells to other places). In absorption, this pattern can be condensed from the perspective of moving things in—not out of—the foods.¹¹

ANATOMICAL BASIS OF ABSORPTION

The total quantity of fluid that must be absorbed by the GI tract each day is equal to the ingested fluid (about 1.5 L in man) plus that secreted in the various gastrointestinal secretions (about 7 L). All but about 1.5 L of the total is absorbed in the small intestine, leaving only these 1.5 L to pass into the colon each day.

The stomach is a poor absorptive area of the gastrointestinal tract because it lacks the typical villus type of increased absorptive membrane surfaces and because the junctions between the epithelial cells in the stomach are tight junctions. Generally, only a few highly lipid-soluble substances, such as alcohol and molecules like aspirin can be absorbed in small quantities. The hydrolytic process of digestion and the extreme range of pH values occurring in the stomach environment serve to make this entry route unattractive for many molecules such as proteins.

Mechanisms to provide intestinal absorption of larger molecules (such as proteins and complex carbohydrates) are very limited in the intestines—the principal path to absorption of these molecules is first metabolism—to break large molecules down to smaller entities that can then be absorbed.⁴¹

However, as discussed later in this chapter, some such molecules (such as botulinum toxin, with molecular weights up to 400,000) can be absorbed into the lymphatic systems.

In the last 15 years, a new area of concern (absorption of novel molecules) has arisen from biotechnology derived foods.

Absorptive Surface of the Intestinal Mucosa—The Villi

The absorptive surface of the intestinal mucosa has many folds called valvulae connivents which increase the surface area of the absorptive mucosa about threefold.⁵¹ These folds extend circularly most of the way around the intestine and are especially well developed in the duodenum and jejunum, where they protrude markedly into the lumen.

Located over the entire surface of the small intestine, from about the point at which the common bile duct empties into the duodenum down to the ileocecal valve, are literally millions of small villi, which project about 1 mm from the surface of the mucosa. These villi lie so close to one another in the upper small intestine that they touch most areas, but their distribution is less profuse in the distal small intestine. The presence of villi on the mucosal surface enhances the absorptive area an additional ten-fold.

Finally, each intestinal epithelial cell is characterized by a brush border, consisting of as many as 1000 microvilli 1 μm in length and 0.1 μm in diameter protruding into the intestinal chyme. This increases the surface area exposed to the intestinal materials at least another 20-fold. The combination of the folds of Kerckring, the villi, and microvilli increases the absorptive area of the mucosa perhaps 1000-fold, in man yielding a tremendous total area of 250 or more square meters for the entire small intestine.

The general organization of the villus, emphasizing especially the advantageous arrangement of the vasculature system for absorption of fluid and dissolved material into the portal blood and the arrangement of the central lacteal for absorption into the lymph, which are pinched-off portions of infolded enterocyte membrane that contain inside the vesicles extracellular materials that have been entrapped. Minute amounts of substances are absorbed by this physical process of pinocytosis, although comparatively these are a very, very small proportion of total absorption. Also, extending linearly into each microvillus of the brush border are multiple actin filaments that contract intermittently and cause continual movements of the microvilli, keeping them constantly exposed to new quantities of intestinal fluid.

BASIC MECHANISMS OF ABSORPTION

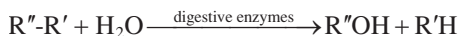
Absorption through the gastrointestinal mucosa occurs by active transport, diffusion, and by solvent drag. Briefly, active transport imparts energy to the substance as it being transported for the purpose of concentrating it on the other side of the membrane or moving it against an electrical potential. On the other hand, transport by “diffusion” means simply transport of substances through the membrane as a result of molecular movement along an electrochemical gradient. Transport by solvent drag occurs anytime a solvent is absorbed because of physical absorptive forces. The movement of the solvent will “drag” dissolved substances along—the basis of many formulation approaches for pharmaceuticals.

Absorption Mechanisms

There are five different mechanisms by which materials can pass from the mucosal side of the GI tract across to the serosal side: passive diffusion through a lipid membrane; diffusion through pores; active energy-dependent transport; absorption through lymphatics; and absorption of macromolecules by pinocytosis.

Hydrolysis as the Primary Step in Absorption of Macromolecules

Almost all carbohydrate xenobiotics are large polysaccharides or disaccharides, which are combinations of monosaccharides bound to one another by condensation. The two monosaccharides then are combined with each other at these sites of removal, and the hydrogen and hydroxyl ions combine to form water. When the carbohydrates are broken down into monosaccharides, specific enzymes return the hydrogen and hydroxyl ions to the polysaccharides and thereby separate the monosaccharides from each other. This process called hydrolysis is the following (in which R''-R' is a disaccharide):



Almost the entire lipid portion of the diet consists of triglycerides (neutral fats), which are combinations of three fatty acid molecules condensed with a single glycerol molecule. In condensation, three molecules of water have been removed. Digestion of the triglycerides consists of the reverse process, the fat-digesting enzymes returning molecules of water to the triglyceride molecule and thereby splitting the fatty acid molecules away from the glycerol. Here again, the digestive process is one of hydrolysis.

Finally, proteins and peptides are formed from amino acids that are bound together by peptide linkages. In this linkage, a hydroxyl ion is removed from one amino acid and a hydrogen ion is removed from the succeeding one; thus, the amino acids in the peptides or protein chain are bound together by condensation, and tend to reverse the process. Proteolytic enzymes return water to the molecules to split them into their constituent amino acids.

Therefore, the chemistry of digestion and the break down of xenobiotics for absorption in the GI tract is simple because in the case of all three major types of primary organic structures, the basic process of hydrolysis is involved. The only difference lies in the enzymes required to promote the reactions for each type of molecule.

Absorption of Water

Water is transported through the intestinal membrane entirely by diffusion, obeying the usual laws of osmosis. Therefore, when the stomach contents are dilute, water is absorbed through the intestinal mucosa into the blood of the villi by osmosis.

At the same time, water can also be transported in the opposite direction, from the plasma into the contents. This occurs especially when hyperosmotic solutions are discharged from the stomach into the duodenum. Sufficient water usually is rapidly transferred by osmosis to make the chyme isosmotic with the plasma. Generally, nature adheres a vacuum.

As dissolved substances are absorbed from the lumen of the gut into the blood, absorption tends to decrease the osmotic pressure of the GI tract contents (or “chyme”). However, water diffuses so readily through the intestinal membrane (because of large 0.7- to 1.5-nanometer paracellular pores through the so called tight junctions between the epithelial cells) that it almost instantaneously “follows” the absorbed substances into the blood. Therefore, as molecules are absorbed, so also is an isosmotic equivalent of water absorbed.

Absorption of Ions

Twenty to thirty grams of sodium are secreted into the intestinal secretions each day. In addition, the normal person eats 5–8 g of sodium each day. Combining these, the small intestine must absorb 25–35 g of sodium each day, which is equal to about one seventh of all the sodium present in the body. Therefore, one can well understand that whenever intestinal secretions are lost to the exterior, as in extreme diarrhea, the body’s sodium reserves can be depleted to a lethal level within hours. Normally, however, less than 0.5% of the intestinal sodium is lost in the feces each day because of its rapid absorption through the intestinal mucosa. Sodium also plays an important role in the absorption of sugars and amino acids.

The motive power for the sodium absorption is provided by active transport of sodium from inside the epithelial cells through the basal and side walls of these cells into the paracellular spaces. This active transport obeys the usual laws of active transport: it requires energy, and the energy process is catalyzed by appropriate adenosine triphosphatase enzymes in the cell membrane. Part of the sodium is absorbed simultaneously with chloride ions; the chloride ions are passively “dragged” along by the positive electrical charges of the sodium ion. Additional sodium ions are absorbed while either potassium or hydrogen ions are transported in the opposite direction in exchange for the sodium ions.

Absorption in the Large Intestine: Formation of the Feces

About 1500 mL of chyme normally pass into the large intestine each day. Most of the water and electrolytes in the chyme are absorbed in the colon, usually leaving less than 100 mL of fluid to be excreted in the feces. Essentially all the ions are absorbed, leaving only 1–5 milliequivalents, each of sodium and chloride ions to be lost in the feces. Most of the absorption in the large intestine occurs in the proximal one half of the colon, whereas the distal colon functions principally for storage.

The mucosa of the large intestine also has a high capability for active absorption of sodium, and the electrical potential created by the absorption of the sodium causes chloride absorption as well. The tight junctions between epithelial cells of the

large intestinal epithelium are much tighter than those of the small intestine. This prevents significant amounts of back-diffusion of ions through these junctions, thus allowing the large intestinal mucosa to absorb sodium ions far more completely than can occur in the small intestine. This is especially true when large quantities of aldosterone are available to enhance the sodium transport capability.

In addition, as in the distal portion of the small intestine, the mucosa of the large intestine secretes bicarbonate ions while it simultaneously absorbs an equal number of chloride ions in an exchange transport process. The bicarbonate helps neutralize the acidic end products of bacterial action in the colon. The absorption of sodium and chloride ions creates an osmotic gradient across the large intestinal mucosa, which in turn enhances absorption of water.

Human's large intestine can absorb up to 5–7 L of fluid and electrolytes each day. When the total quantity entering the large intestine exceeds this amount, the excess appears in the feces as diarrhea. As noted earlier, toxins from cholera, other bacterial infections, and certain other xenobiotics can often cause the crypts of Lieberkühn in the terminal ileum and the large intestine to secrete more liters fluid each day, leading to severe and sometimes lethal diarrhea.

Numerous bacteria, especially colon bacilli, are normally present in the absorbing colon. They are capable of digesting small amounts of cellulose, in this way providing a few calories of nutrition to the body each day. In herbivorous animals, this source of energy is significant, although it is of negligible importance in human beings. Other substances formed as a result of bacterial activity are vitamin K, vitamin B₁₂, thiamin, riboflavin, and various gases that contribute to flatus in the colon, especially carbon dioxide, hydrogen gas, and methane.⁷⁷ Vitamin K is especially important because the amount of this vitamin ingested in foods is normally insufficient to maintain proper blood coagulation.

The feces normally are about three-fourths water and one-fourth solid matter which is itself is composed of about 30% inorganic matter, 2%–3% protein, and 30% undigested roughage of the food and dried constituents of digestive juices, such as bile pigment and sloughed epithelial cells. The large amount of fat derives mainly from fat formed by bacteria and fat in the sloughed epithelial cells. The brown color of feces is caused by stercobilin and urobilin, derivatives of bilirubin. The odor is caused principally by the products of bacterial action; these products vary from one individual or species to another, depending on colonic bacterial flora and on the type of food eaten.

Effects of pH and Volume

A number of barriers are present to impede the simple diffusion of molecules across the membranes of the epithelial cells lining the tract. First, the molecules must transverse the unstirred layers of fluid lying immediately adjacent to the membrane, then cross the mucus layer coating the membrane and finally cross the bilipid membrane itself into the cell. Once within the cytoplasm of the cell, the molecule must pass through the cytoplasm and then through the basement membrane and the capillary or lymphatic wall membranes. The bilipid structure of the cell membrane

greatly favors the absorption of hydrophobic structures over hydrophilic ones. The fact that ionized forms are marginally absorbed may be the result of microenvironments of lower pH in the unstirred layers immediately adjacent to the membrane, or to the acidity of the membrane itself.

The process of diffusion is driven by the concentration gradient across the membrane, and thus the fluid volume in the tract can have a major influence on the rate at which an ingested material may appear in the bloodstream. The active transport of sodium through the basolateral membranes of the cell reduces the sodium concentration inside the cell to a low value. Sodium moves down a steep electrochemical gradient from the chyme through the brush border of the epithelial cell into the epithelial cell cytoplasm, replacing the sodium that is actively transported out of the epithelial cells into the paracellular spaces.

Next, water moves by osmosis into the paracellular spaces. This is caused by the gradient created by the elevated concentration of ions in the paracellular space. Most of this osmosis occurs through the tight junctions between the apical borders of the epithelial cells, but a smaller proportion occurs through the cells themselves. The osmotic movement of water creates a flow of fluid into and through the paracellular space and from there to the circulating blood of the villus.

When a person or animal becomes dehydrated, large amounts of aldosterone are almost always secreted by the adrenal glands. Within 1–3 hours the excess aldosterone greatly enhances all the enzyme and transport mechanisms for all aspects of sodium absorption by the intestinal epithelial cells. The increased sodium absorption then causes secondary increases in absorption of chloride ions, water, and some other substances. Aldosterone in the intestinal tract acts the same as that activated by aldosterone in the renal tubules, which also serves to conserve salt and water in the body when an organism becomes dehydrated.

In the upper part of the small intestine, chloride absorption is rapid and mainly by passive diffusion. The absorption of sodium ions, through the epithelium creates slight electronegativity in intestinal chyme and electropositivity on the basal side of the epithelial cells. Chloride ions move along this electrical gradient to “follow” the sodium ions.

Often large quantities of bicarbonate ions must be reabsorbed from the upper small intestine because of the large amounts of bicarbonate ions in both the pancreatic secretion and the bile. The bicarbonate ion is absorbed individually as follows: When sodium ions are absorbed, moderate amounts of hydrogen ions are secreted into the gut in exchange for some of the sodium, such hydrogen ions in combine with the bicarbonate ions to form carbonic acid (H_2CO_3), which then dissociates to form water and carbon dioxide. The water remains in the intestines, but the carbon dioxide is readily absorbed into the blood and subsequently expired through the lungs. This is a so-called active absorption of bicarbonate ions. It is the same mechanism that occurs in some of the tubules of the kidneys.

Effects of Xenobiotics on Microbiome

Digestion (and absorption) of xenobiotics depends to a significant degree upon the microorganism population resident in the intestines. While the effects of antibiotics

have long been recognized, the effects of a much broader range of chemical entities and physical factors (such as radiation) have only recently been appreciated.

Secretion of Bicarbonate Ions in the Ileum and Large Intestine—Simultaneous Absorption of Chloride Ions

The epithelial cells on the surfaces of the villi in the ileum as well as on all surfaces of the large intestine have special capability of secreting bicarbonate ions in exchange for absorption of chloride ions. This provides alkaline bicarbonate ions that are used to neutralize acid products formed by bacteria, especially in the large intestine. The exact mechanism of this exchange is unclear but depends on the exchange of protein in the luminal membrane of the epithelial cell that forcibly exchanges bicarbonate ions formed inside the cell for chloride ions in the intestinal lumen. The excess chloride in the cell is then transported by facilitated diffusion through the basolateral membrane of the epithelial cell, completing the chloride absorption.

Immature epithelial cells in the crypts of Lieberkühn continually divide to form new epithelial cells that then spread outward over the luminal surfaces of the intestines. These new cells have properties different from those of the mature cells already on the outer luminal surfaces. Normally, they secrete small quantities of sodium chloride and water into the intestinal lumen, but this secretion is immediately reabsorbed by the older epithelial cells outside the crypts, providing a watery solution for absorbing intestinal digestates.

Extreme secretion is initiated by entry of a subunit of the cholera toxin into the cell. This stimulates the formation of excess cyclic adenosine monophosphate, which then opens tremendous numbers of chloride channels, allowing chloride ions to flow rapidly from inside the cell into the crypts. In turn, this is believed to activate a sodium pump that pumps sodium ions into the crypts to go along with the chloride ions. Finally, all this extra sodium chloride causes extreme osmosis of water into the crypts as well, thus providing the rapid flow of fluid along with the salt. Initially, all this excess fluid washes away the bacteria and is of value in combating the disease, but ultimately can be lethal because of the serious dehydration of the body that may ensue.

The transport of an absorbed molecule into the bloodstream is driven by a large concentration gradient between the luminal side and the serosal side of the intestine. Because of the rapid blood flow and concomitant rapid removal of absorbed solutes into the general systemic circulation, concentration gradients are invariably favorable for movement from the gut into the circulatory system. The major impediment to absorption of nutrients and exogenous chemicals is the initial movement across the mucosal cell membrane.

It is believed that a major portion of the water present in the GI tract is reabsorbed through pores that are present in the apical junctions of the epithelial cell lining. These pores are large enough to allow penetration of small molecules, particularly small ionized species. The net direction of flow of water is either from the GI tract into the serosal fluid, as is generally the case when the contents are

either iso-osmotic or hypotonic, or into the GI tract, as might occur when the gastro-intestinal contents are hyper-osmotic or in certain pathological states. Vogel et al.⁷⁰ studied the effect of water flow on the toxicity of atropine, an azoniaspiro compound, phenobarbital, and nicotine by infusing solutions of these substances into the duodenum of rats. Mannitol was concomitantly infused to adjust the osmotic concentration of the contents and thus the flow of water from the serosal side to the mucosal side of the GI tract. Toxic effects were increased by a factor of 2–4 with a decrease in osmotic concentration from triple isotonicity to isotonicity for three of the four materials, the only exception being with the azoniaspiro compound. The depression of absorption of solutes resulting from the flow of water from the serosa into the lumen of the intestine has been termed solvent drag. This process can play an important role in evoking toxic responses of certain compounds, primarily small water-soluble ions.

Active Transport

Active energy-dependent transport mechanisms of absorption are primarily for molecules resembling nutrients, i.e., amino acids, sugars, essential vitamins and minerals, etc. Specific exogenous substances can also be absorbed from the GI tract using these same transport systems. For instance, pyrimidines and amino acids are absorbed by active transport systems. 5-Fluorouracil and 5-bromouracil are actively transported across the rat intestinal epithelium by the process which transports natural pyrimidines,⁶⁵ and penicillamine and levodopa utilize an active transport mechanism for natural amino acids. Processes designed for the transport of essential metals are also responsible for the absorption of such toxic metals as lead and aluminium. Chlorothiazide has been demonstrated to be absorbed by a non-saturable active absorption process.⁷⁹

MAMMALIAN ABSORPTION TRANSPORTERS

Absorption is not purely a matter of passive mechanisms based on physiochemical characteristics of a xenobiotic. There are also active mechanisms by which molecules are taken into the body. In the GI tract, there is a whole family of active “transporter” mechanisms.⁵⁶ All of the below are present in humans and most common laboratory species to varying extents.

Peptide Transporter

- Located in the brush border membrane of the intestine.
- Broad substrate specificity of various peptidomimetic drugs and small peptides.
- Many β -lactam antibiotics and some other drugs can be transported via this system, e.g., cephalexin, ampicillin, amoxicillin, captopril (and other ACE inhibitors), and the amino acid prodrug of acyclovir (has no peptide bond, suggesting a broader spectrum than just peptides).

Nucleoside Transporter

- One type involved facilitated diffusion (carrier attachment without energy input)
- Another type involved in active transport (requires input of energy)
- Probably affects absorption of nucleoside analogues used in antiviral and anti-cancer therapies

Sugar Transporter

- There are at least two types with high affinities for D-glucose and D-fructose and low affinities for D-galactose and mannose. At least one type is facilitated transport.
- L-Sugars have an affinity about 1000 times less than D-sugars.

Bile Acid Transporter

- Preserve bile salts via absorption from the ileum (enterohepatic recirculation)

Amino Acid Transporters

- Many different types exist in the intestine
- Some drugs absorbed with these transporters include α -methyl dopa, baclofen, and D-cyclosporin.

Organic Anion Transporters

- Anions are generally negatively charged moieties, such as produced by carboxylic acids in neutral or basic media, e.g., acetate.

Vitamin Transporters

- The following have specific transporters: thiamine, vitamin C, folic acid & vitamin B-12.
- Methotrexate (analogue of folic acid) is absorbed by the intestine in a manner similar to folic acid.
- The nicotinic acid transporter also has affinity for valproic acid, salicylic acid, and penicillins.

Phosphate Transporter

- Foscarnet (antiviral drug) and fosfomycin (water-soluble antibiotic) utilize this transporter.

Bicarbonate Transporter

- These are the principle regulators of pH in cells, and play a vital role in acid-base movement.
- These transporters help exchange bicarbonate, sodium, and chloride.

Organic Cation Transporter

- Choline is a substrate.

Fatty Acid Transporter

- Long chain fatty acids such as palmitate and oleate can saturate the transporter, but not short-chain fatty acids.
- A protein may assist in directing fatty acids to transporter sites.

Human Efflux Transporters (P-Glycoprotein)

- Exists on the brush border.
- Pumps out (exorbs) a large number of drugs (broad substrate specificity).
- Substrates include vincristine, taxol, digoxin, some fluoroquinolone antibacterials, quinidine, etoposide, cyclosporine, varapamil, and nifedipine.⁶⁶

ABSORPTION BY LYMPHATICS

Absorption by way of the lymphatics is limited to nonpolar materials and operates by mechanisms analogous to that which absorbs fatty acids. Bile salts play an important role in dispersing triglycerides and other fat-soluble molecules and are critical in the formation of micelles that allow dissolution of the fatty materials within the chyme. The fact that rats do not possess a gall bladder may lead to differences in their ability to absorb materials efficiently by way of the lymphatics. Fatty acids, derived from the hydrolysis of triglycerides by various lipases, migrate to the brush border of the mucosal cells and readily diffuse through the mucosal membrane into the cytosol of the cell. Once in the cell, the fatty acids are reincorporated into triglycerides within the endoplasmic reticulum and packaged into chylomicrons, a conglomerate of triglycerides, cholesterol, and phospholipids encased in a protein coat. These provide an ideal environment to entrain other lipid-soluble molecules. The protein coat provides a hydrophilic exterior to the conglomerate that is extruded from the cell into the serosal fluid and into the central lacteals of the villi. The chylomicrons are pumped through the lymphatic system and empty into the systemic circulatory system at the entrance of the thoracic duct in the veins of the neck. In this manner, fatty lipophilic materials avoid entering the hepatic portal circulatory system and first-pass effects of metabolism by liver enzymes. Sieber⁶⁷ has shown that p,p'-DDT and structurally related analogues are absorbed through the lymphatics. The extent of lymphatic absorption is limited, however, presumably because of the relatively slow rate of movement of lymph through the lymphatics as compared with movement of blood through the general circulatory system. The extent of absorption may vary greatly depending on the vehicle in which a test substance is administered. Thus, Sieber⁶⁷ recovered only 15% of a dose of p,p'-DDT in the lymph when administered in ethanol compared with 34% when administered in corn oil.

P-Aminosalicylic acid (PAS) and tetracycline have also been demonstrated to be absorbed by way of the lymphatics. Both of the silicone oils, drugs are also rapidly distributed throughout the extracellular fluid, including lymph, when administered by the intravenous route.¹⁹ Thus, care must be taken in interpretation of data in which accountability of a substance in lymph is used to determine absorption through lymphatics after peroral dosing. Other materials shown to be absorbed by way of the lymphatics include 3-methylcholanthrene, polychlorinated biphenyls, and benzpyrene.

Macromolecules

The direct absorption of macromolecules from the GI tract is well established and has both wide application in pharmacotherapeutics and grave toxicological implications in some instances, e.g. the absorption of botulinum toxins, which are proteins of molecular weight ranging from 200,000 to 400,000.⁷⁶ Macromolecules are believed to be absorbed by pinocytosis. Intestinal mucosas in the area of the Peyer's patches and lymphoid follicle aggregates are believed to be particularly active in this respect.¹ Peyer's patches are the site of lymphoid follicle-associated epithelium which contain cells capable of transporting antigens and microorganisms.²⁶ Mucosa-associated lymphoid tissue is separated from the lumen of the intestine by lymphoid follicle-associated epithelium.

The ability of macrostructures to cross the epithelium lining, particularly in the area of Peyer's patches, has been explored for use in the delivery of pharmaceutical and molecular biological preparations. Polyanhydride copolymers of fumaric and sebacic acid have demonstrated high biological adhesive properties.⁴⁵ Microspheres of this copolymer with diameters ranging from 0.1 to 10 μm have been fed to rats observed by histological procedures to transverse both the mucosal epithelium, through and between individual cells, and the follicle-associated epithelium covering the lymphatic elements of Peyer's patches.

Once taken into the mucosal cells, the macromolecules are transported to the general circulation by way of the lymphatics. The process is age dependent, decreasing with age.

ACQUISITION OF PASSIVE HUMORAL IMMUNITY POSTNATALLY

We should at this point recall that the GI tract serves not only as one of the three principal routes for entry of xenobiotics into the body for mammals, but also as one of the major routes for immune system interaction with the environment. There are two major avenues available for the absorption of macromolecules, such as immunoglobulin G (IgG), by the intestine. One is receptor mediated endocytosis and the other is non-receptor-mediated endocytosis.

Coated pits and vesicles are involved in receptor-mediated endocytosis.^{17,50} Clathrin, with a molecular weight 180,000, is the major structural protein of coated pits and vesicles.^{50,54} Clathrin defines the cytoplasmic side of

endocytotic organelles by enclosing them in a lattice of pentamers and hexamers. Finally, a polyhedral vesicle is produced. In receptor-mediated endocytosis, it is believed that specific protein receptors are randomly dispersed in the lipid bilayer of the plasmalemma.⁴⁹ To initiate internalization, the macromolecule or the ligand binds to its receptor. This in turn promotes the binding of clathrin to the other end of the receptor; next, ligand-receptor-clathrin complexes cluster at the base of the microvillus, causing the plasmalemma to invaginate or pit. The coated pit pinches off forming a coated submicroscopic vesicle that then traverses the cell and fuses with the basal lateral membrane. Finally, the microvesicle undergoes reverse pinocytosis (exocytosis), resulting in the discharge of the ligand into extracellular space.^{70,72}

In nonreceptor-mediated endocytosis, macromolecules are trapped within a submicroscopic apical tubular system, which is contiguous with the plasmalemma.^{70,72} The trapped macromolecules flow down the tubule, eventually reaching the apical cytoplasm. The blind end pinches off and forms a micro vesicle, visible by light microscopy. These macro vesicles join lysosomes, becoming phagolysosomes. Intracellular digestion is the most probable fate of macromolecules internalized by this nonselective route.

Another mechanism exists for transporting macromolecules (soluble antigens, bacteria, viruses, etc.) through endocytosis.^{10,52,53,75} M cells (specialized cells sandwiched between enterocytes overlying Peyer's patches) may be responsible for initiating a local immune response by transferring g antigens from the gut lumen to the lymphoid tissue in the Peyer's patches.

When Do Mammals Acquire Passive Humoral Immunity?

Mammals can be divided into three groups based on when they acquire passive humoral immunity.^{8,28} Group I mammals acquire passive immunity exclusively postpartum; some examples are pigs, horses, and ruminants. Group II mammals acquire passive immunity both pre- and postpartum; examples are mice, rats, hamsters, dogs, and cats. Group III mammals acquire passive immunity exclusively prepartum and include humans, other primates, and guinea pigs.

Neonates in Group I nonselectively absorb macromolecules for a short period postpartum. Enterocytes on the intestinal epithelium of neonates in Group I nonselectively transport macromolecules from the lumen of the gut into the blood for about 2 days postpartum.^{2,8,9,28,31,35} However, enterocytes continue to internalize (but do not transport) macromolecules nonselectively for a much longer period. Because of the dichotomy between internalization and transport, the absorption of macromolecules is divided into two phases: (a) uptake or internalization within the enterocyte and (b) transport through the enterocyte.

The period after which enterocytes can no longer internalize macromolecules is called "closure."^{9,31,32,36} In the neonatal pig, dietary regimens influence both cessation of transport and closure.^{25,27,31,32,36,37} Piglets denied food from birth transport macromolecules from the gut into the blood for as long as they live (about 3.5 days). While, nursing pigs eating about 300 mL of colostrums or milk cease

transporting within 24 hour postpartum.³⁷ It appears as though eating stimulates the release of humoral signal (hormone), which turns off transport. Although transport is qualitatively nonselective, the process is energy-dependent²⁹ and preferential; i.e., more immunoglobulin is transported than albumin when ligated segments of neonatal gut are injected with a solution containing both albumin and IgG. Also, the upper half of the small intestine transports more efficiently than the lower half. And, enterocytes in the lower half have more lysosomal-like proteolytic activity.²⁷

By the time the pig has nursed for 2 days, the intestine can no longer transport macromolecules; however, the lower half of the small intestine can still internalize them. As the pig ages, more and more of the small intestine (proceeding toward the ileum) ceases internalizing macromolecules. Finally, by 2–3 weeks of age all enterocytes, throughout the length of the small intestine, are closed and the pig cannot internalize macromolecules.³¹ As with transport, dietary regimens affect the time of closure. And, the signal for closure again seems to be humoral, since an intestinal segment surgically removed from the digestive pathway, as above, closes at the same time as its counterpart in the digestive pathway.²⁴

Histological studies show that enterocytes capable of transporting macromolecules have a submicroscopic noncoated interconnecting tubule system that traverses the enterocytes from plasma lemma to the basal lateral membrane.²² These kinds of enterocytes are found mainly in the upper third of the small intestines of newborn pigs. In the lower third of the small intestines of the newborn pigs, enterocytes mainly internalize macromolecules for digestion. These enterocytes have a few apical tubules and many small vacuoles, which appear to fuse, eventually producing a large macroscopic vacuole. Enterocytes in the mid small intestines of newborn pigs contain numerous apical tubules and vacuoles, indicating that they are both internalizing and transporting macromolecules.

Thus, the neonatal pig has enterocytes that transport macromolecules (mainly upper small intestines), enterocytes that internalize and digest macromolecules (mainly lower small intestines), and enterocytes that do both (mainly mid small intestines). At about 2 days of age, neonatal pigs cease transporting macromolecules from the gut lumen to the blood. They cease internalizing macromolecules in the upper half of the small intestines. They continue to internalize nonselectively macromolecules in the ileal area for 2 more weeks. Both time of cessation of transport and closure are influenced by a humoral signal induced by eating. The separation of transport from digestion would be of value to a neonate requiring intact protein for transport (IgG) and digested protein for amino acid building blocks.

Animals in Group II selectively absorb macromolecules for an extended period of time, e.g., rats for 21 days, mice for 17 days, and hamsters for 7 days postpartum.^{8,30,32}

Rats, mice, and the hamster have been extensively studied regarding this phenomenon, absorption can be divided into a transport in rats and mice occurs exclusively in the upper third of the small intestines and is selective for IgG.^{30,42,57} The lower third of the small intestine nonselectively internalizes macromolecules and the mid third does both.

Clathrin-like coated pits, coated vesicles, and Fc-receptors for IgG have been associated with an apical tubular system in enterocytes located in the upper part of the small intestines but not with the apical tubular system in the enterocytes in the ileal area.^{40,57,70,72,73} Thus, it seems that IgG is routed through the enterocyte of the upper gut in coated submicroscopic vesicles^{70,72} and tubules to the basal lateral membrane, a process not too different from the pig except that the neonatal rat's enterocytes absorb selectively via clathrin-coated organelles.

Enterocytes in the lower part of the small intestine internalize macromolecules nonselectively in uncoated submicroscopic vesicles that fuse with others. Eventually, as with the ileal area in the pig, these fusing vesicles produce a macroscopic vesicle that appears to join the supranuclear vacuole.⁷⁰ Thus, the internalization of macromolecules in the ileal area leads to the digestion or storage of the macromolecules rather than transport. Compartmentation is an elegant way to reconcile the neonate's distinct needs for intact immunoglobulin for transport into the blood and digested proteins for building blocks. Cessation of transport and closure occur at the same time, coincident with weaning. These phenomena may be under adrenal hormone control in that glucocorticoids (at unphysiologically high levels) can precociously initiate closure provided the neonate's adrenals have reached the proper maturational state.^{21,43,44}

Neonates in Group III lack the capacity to transport macromolecules postnatally in an appreciable amount.⁸

Even though neonates in this group do not transport macromolecules in appreciable amounts, perhaps they can internalize and digest macromolecules for a short period. This notion is supported by evidence from research using guinea pigs. Guinea pigs can nonselectively internalize macromolecules for about 2 days postnatally.^{32,58} Pinocytotic macrovesicles are present in enterocytes during the internalization phase and probably contribute to the digestion of soluble protein in this early neonatal period.³² Thus, enterocytes in the neonatal guinea pig have internalizing and digesting organelles that are similar to those seen in the neonates in categories II and I.

There are reports of small amounts of antibody transported by the nursing human infant's gut.^{5,20} Interestingly, the human infant's gut during fetal development undergoes maturational changes analogous to those seen postnatally in pigs and rats.^{3,39,46-48} These structural changes are apical tubules that appear in enterocytes throughout the entire length of the small intestine at the time villi are formed, around 10 weeks of gestation. (This same kind of structure is seen in the suckling rat and newborn pig as noted above.) Apical tubules and lysosomal elements are more numerous in the lower third of the intestine (again, like the suckling rat and pig). By 22 weeks gestation, apical tubules disappear, and enterocytes in the upper area of the small intestine resemble adult-type enterocytes (a pattern seen in a 2- to 8-day-old pig). At term, or shortly thereafter, the infant's gut is replete with adult-type enterocytes. Although fetal enterocytes in the human seem capable of internalizing macromolecules, there is some doubt regarding the capacity of these enterocytes to transport macromolecules through the cell in an appreciable amount.⁴⁸

ADULTS

Normal

Investigators using a highly sensitive enzymatic macromolecular marker (horseradish peroxidase) have found evidence for transport of this macromolecule through adult rat and rabbit enterocytes.^{12,18,61,69} Horseradish peroxidase injected into ligated segments of adult jejunum was visualized histochemically within enterocytes in an apical tubular system. Further, this marker was transmitted into the extracellular space of the lamina propria. Others, using horseradish peroxidase and lactoperoxidase, have detected an apical tubular system in enterocytes that are on villi in organ-cultured adult human small intestine.⁵⁻⁷ Adult enterocytes internalize macromolecules in a manner analogous to that described for the neonatal rat except that the apical tubular system is less well developed in the adult.^{12,61,69}

Thus, there is evidence that low levels of macromolecules (antigens) can break the adult mucosal barrier. They can be absorbed nonselectively by enterocytes via an apical tubular system (which may be a vestige of the neonatal system) or they can be absorbed by M cells.

Abnormal

In the normal adult, low-level absorption of macromolecules does not seem to be a threat to health.^{60,62} However, disease could result if increased quantities of antigens or toxic substances were absorbed. For example, in adults with gastric and pancreatic insufficiency, macromolecules are less efficiently digested. Thus, higher concentration of macromolecules would be presented to the enterocytes. Therefore, more macromolecules would likely be absorbed.^{23,59,60,62,68} Also, an increase in absorption would occur if intracellular digestion was decreased because of faulty lysosomes. Mucosa that no longer functions as a viable structure (radiation damage) could serve as a source of the passively transmitted macromolecules. Increased absorption of macromolecules could result from an immune deficiency. In this case, secretory antibodies capable of reacting with antigens and thereby blocking their absorption would be lacking.^{14,16,63,64} Certain diseases—e.g., celiac disease, allergic gastroenteropathies, inflammatory bowel disease, viral and bacterial enteritis, parasitic infestations of the gut, and radiation enteritis—may be associated with increased absorption and transport of macromolecules.^{4,60,62}

CONCLUSION

Neonatal mammals have ready mechanisms for absorbing macromolecules. The normal function of these mechanisms is to absorb physiologically useful macromolecules such as immunoglobulins. However, if the neonate is ill-managed and placed in an environment replete with toxic macromolecules, then the potential exists for absorbing these toxins. In the neonatal period when the pig and mouse have an immature intestinal epithelium, they are more susceptible to enteric pathogens like

Escherichia coli and rotavirus, and they can internalize toxins like endotoxins from gram-negative rods.^{13,33,34,38,55,74} As the intestine matures and can no longer absorb macromolecules, these animals become less susceptible to these enteropathogens.

A remnant of the neonatal system for absorbing macromolecules remains in mature mammals. Thus, adults can absorb macromolecules at a low level. In the physiologic normal mature adult, the absorption of insignificant quantities of macromolecules produces no ill effects. However, if alteration in the intraluminal digestive process occurs or if the mucosal barrier becomes defective, then increased quantities of antigenic or toxic substances could gain entrance to the body, resulting in local intestinal or systemic disorders.

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CHAPTER 9

Peyer's Patch Epithelium

An Imperfect Barrier and Effect of the Microbiome

Gary R. Burleson and Florence G. Burleson

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GI TRACT: DEFINITION

Morphologically, the gastrointestinal (GI) tract can be described as a tube extending from the oral cavity to the anus. The longest section of the GI tract is the small intestine that connects the pylorus of the stomach to the large intestine or colon. The small intestine is divided into three sections: the duodenum, the jejunum, and the ileum. The GI tract consists of four distinct layers extending from

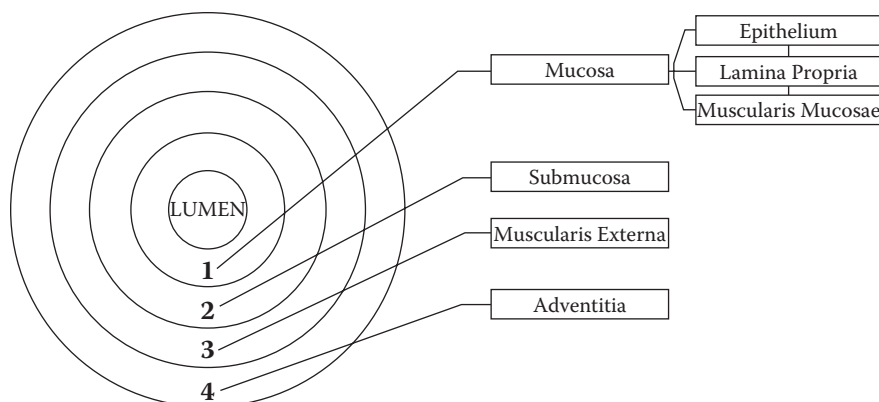


Figure 9.1 Diagram of the four layers of the gastrointestinal tract.

the mucosa (adlumina or innermost layer closest to the lumen) to the adventitia or serosa or most abluminal or outermost layer from the lumen ([Figure 9.1](#)). These four layers include: (1) the mucosa which includes the epithelium, lamina propria, and muscularis mucosae, (2) the submucosa which includes the connective tissue, (3) the muscularis externa which is the muscular wall, and (4) the adventitia or serosa layer (see [Figure 9.1](#)). The mucosal epithelium lines the entire gastrointestinal tract and is characterized by folds resulting in crypts and villi that increase the surface area for nutrient absorption which is one of the major functions of the GI tract and performed by enterocytes. The crypts or invaginations, also known as the crypts of Lieberkühn, are the primary site for the location of Paneth cells. Paneth cells are specialized secretory cells that can produce antimicrobial peptides. The mucosa is the most highly differentiated layer of the gastrointestinal tract. The mucosal epithelium includes the apical surface (gut luminal side) and the basal or connective tissue side. The gastrointestinal differentiation is related to function along the gastrointestinal tract and includes: protective aspects of the epithelium, production of digestive acid and enzymes, mucus-secreting cells, absorption of nutrients, secretory epithelium, stem cells for replenishment, smooth muscle fibers, and lymphoid cells. Specialized functions include sensory discrimination, mechanical processing, enzymatic and chemical digestion, and immunological surveillance. Discrimination between pathogens and commensal bacteria is provided by an interaction of lymphoid cells with the intestinal epithelium (Jung et al., 2010). The gut-associated lymphoid tissue (GALT) is made up of both isolated and aggregated lymphoid follicles (Neutra et al., 2001). These aggregated lymphoid follicles were first described by Marco Aurelio Severino in 1645 in Italy and were named Peyer's patches after the thorough description by the Swiss pathologist Johann Conrad Peyer in 1677 (Jung et al., 2010).

MUCOSAL EPITHELIUM BARRIER FUNCTION

The mucosal epithelium with its tight junctions forms an effective physical barrier that normally functions as a perfect barrier. The cells of the mucosal epithelium form a continuous intact physical barrier with tight junctions formed with each neighboring cell. This tight physical barrier separates the inside of the human body from the outside. Cells of the mucosal epithelium have three important functions: (1) nutritional function (see [Chapters 1 and 8](#)), (2) barrier function (see [Chapters 1 and 8](#)), and (3) immunological function. The mucosal epithelium thus acts as a watchdog, sentinel, or security guard to allow essential nutrients and electrolytes to enter, to deny passage of pathogenic microorganisms, to generate an immune response against pathogenic microorganisms, and to prevent an immune response against nonpathogenic or commensal microorganisms, or to the foods that provide nourishment. However, the complexity of the mucosal epithelium which includes the multitude of tissue layers, cell types, cell functions, cellular control mechanisms, and messenger molecules such as hormones, neurotransmitters, cytokines, and chemokines makes providing the perfect barrier a difficult balancing act to maintain. The mucosal epithelium must perform disparate functions: serve as a barrier to protect the body from the outside world while serving as a mechanism to allow entry of nutrients, electrolytes, water, and antigens in order to allow initiation of an immune response.

The mucosal epithelium of the small intestine is folded into repeated villus and crypt structures and has a surface area of 400 m² (MacDonald and Monteleone, 2005). While this large surface area is beneficial for the absorption of nutrients, water, and electrolytes, this large access area is not beneficial and in fact complicates the immunological function.

MUCOSAL IMMUNITY

The perfect immunological GI barrier would prevent disease from infectious pathogenic microorganisms. Protection from infectious disease would be mediated by (a) a physical barrier to infection, (b) innate immunity, and (c) adaptive immunity. The perfect barrier would prevent an inappropriate immune response including an immune response to commensal microorganisms, food hypersensitivity, Crohn's disease, and IBD. The perfect barrier would allow commensal microorganisms of the normal GI flora to co-exist.

Control mechanisms must not allow an immune response to food antigens or against the commensal microorganisms residing in the intestines as part of the normal flora and also must not allow entry of infectious agents that may result in a serious local or systemic infection. That is, the entry must allow antigenic sampling in order that an immunological response can be initiated and produced in defense against pathogenic microorganisms, but not against commensal microorganisms or

nutrients or food antigens. While the mucosal epithelium performs as the barrier with its tight junctions, the selective entry or antigenic sampling is performed by specialized immune cells in the Peyer's patches.

MALT AND MUCOSAL IMMUNITY OF THE GI TRACT

The mucosa-associated lymphoid tissue (MALT) is used to describe all the mucosal lymphoid tissues in the body. One component of the MALT is the mucosal intestinal immune system that provides immunological surveillance and includes the GALT or gut-associated lymphoid tissue. Resistance to infectious and neoplastic disease is the *raison d'être* of the immunological armamentarium (Burleson, 2000). The total immune system of the body consists of a vast network of lymphoid and nonlymphoid cells, communicating via messenger molecules to create a functional immune system to protect against disease. This network, while interconnected via mediator molecules, contains compartmentalized units especially equipped to defend against insults to the integrity of the immune system (Burleson, 2000). The intestinal immune system, like the pulmonary immune system, is an example of such a specialized and compartmentalized system that, while capable of performing all immunological functions locally, is also capable of interacting with the systemic immune system, as reviewed by Burleson (1987, 1995, 1996, 2000) and Lebrech and Burleson (1994).

Mucosal immunity in the GI tract is composed of physical factors and non-immune molecules, plus non-immune (non-lymphoid cells) and immune (lymphoid) cells of the mucosal epithelium and Peyer's patches.

Physical Factors and Non-Immune Molecules

The physical barrier is provided by the continuous line of epithelial cells to protect against infection, to exclude unwanted macromolecules, and to minimize fluid and electrolyte loss into the intestinal lumen (Figure 9.2). There are several

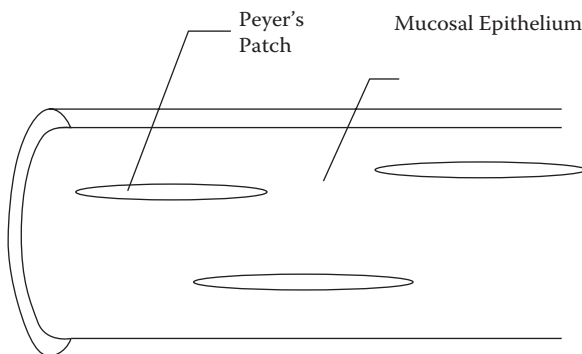


Figure 9.2 Gastrointestinal tract with the mucosal epithelium and Peyer's patches.

components of the physical barrier all designed to keep potential pathogens from reaching the epithelium. Pathogenic microorganisms must compete with microorganisms of the normal flora for nutrients and space. Mucus is secreted by goblet cells that not only binds and immobilizes potential pathogens but also contains antimicrobial substances including lysozyme, hydrolytic enzymes, lactoferrin, and secretory antibodies. The brush border with its glycocalyx containing mucin-like molecules provides a protective shield over the epithelial cells and also immobilizes potential threats to the epithelium (MacDonald and Monteleone, 2005). Peristalsis is another physical mechanism that will move infectious agents along unless the microorganism attaches to a susceptible epithelial cell.

Nevertheless, the gut epithelial barrier does not completely prevent luminal antigens from entering the tissues. Thus, intact food proteins can be detected in plasma (Husby et al., 1985) and a few gut bacteria can be detected in the mesenteric lymph nodes draining the gut of healthy animals (Berg, 1995). Antigens can cross the epithelial surface through breaks in tight junctions, perhaps at villus tips where epithelial cells are shed, or through the follicle-associated epithelium (FAE) that overlies the organized lymphoid tissues of the intestinal wall (Neutra et al., 2001).

Non-Lymphoid Cells

Non-lymphoid cells include the intestinal epithelial cells (IECs) that form a continuous monolayer separating the outside world from the human body (Christ and Blumberg, 1997). IECs are capable of producing defensins such as cryptdins and numerous cytokines as messenger molecules. These messengers call in and activate the effector cells of the immune system. IECs are also involved in IgA transport as well as antigen uptake and presentation (Christ and Blumberg, 1997). Intestinal epithelial cells (IECs), enterocytes (the absorptive intestinal cells), goblet cells, Paneth cells (located at the bottom of intestinal crypts), and enteroendocrine cells that produce neuroendocrine molecules with autocrine and paracrine effects on intestinal cells are each important nonlymphoid cell contributors to immunological protection.

Lymphoid Cells: Mucosal Epithelium

Lymphoid cells residing in the mucosal epithelium consist predominantly of the following cell types (Figure 9.3): intestinal intraepithelial lymphocytes (IELs) including IEL $\alpha\beta$ and IEL $\gamma\delta$, natural killer (NK) cells, and macrophages. These cells are capable of providing both innate (nonspecific) immunity and specific (acquired) immunity.

Peyer's Patches: Definition and Function

Peyer's patches are lymphoid aggregates or nodules that were first described in 1677 by Johanni Conradi Peyer in his thesis entitled "De glandulis Intestinorum Earumque Usu et Affectionibus Cui Fubjungitur Anatome Ventriculi Gallinacei" (Croitoru and Bienenstock, 1994). Peyer's patches are located in the distal ileum of

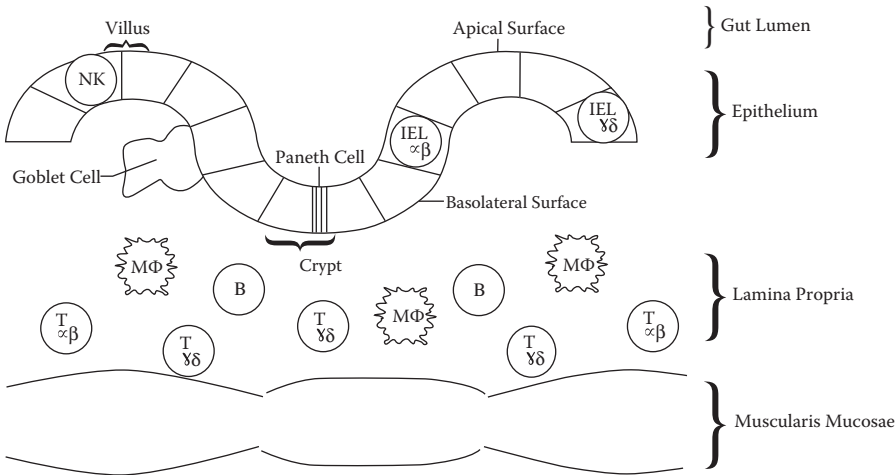


Figure 9.3 Non-immune and immune cells of the mucosal epithelium and lamina propria.

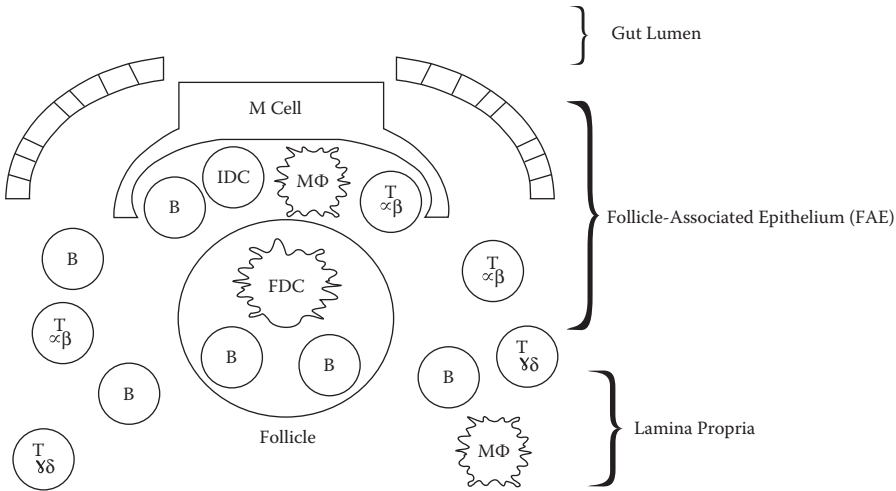


Figure 9.4 M cell, Peyer's patches, and immune cells.

the small intestine. The ileum in addition to the increased number of Peyer's patches or mucosal lymphoid tissue also has more goblet cells than proximal sections of the small intestine.

Immunological surveillance within the mucosal epithelium is primarily provided by Peyer's patches (Figures 9.2 and 9.4). Peyer's patches consist of mucosal lymphoid aggregates that functionally perform the important role of initiating the local immune system. Peyer's patches are a collection of lymphoid tissue underneath the follicle-associated epithelium (FAE) consisting of cells with microvilli.

These lymphoid follicles differ from lymph nodes in the lack of a capsule, lack of a medulla, lack of afferent lymphatic ducts, and lack of a clear border. M cells are situated within this epithelium among the microvilli-covered cells. Also present are B-lymphocytes, macrophages, follicular dendritic cells, and NK cells.

M Cells

Specialized epithelial cells designated membranous or microfold cells (M cells) provide access to Peyer's patches ([Figure 9.4](#)). Antigen attaches and enters M cells that contain a specialized epithelium and are located above the lymphoid follicles. M cells continuously transport intestinal bacteria and antigens from the lumen into lymphoid tissues (Kanoi, 1991). These specialized M cells in the follicle-associated epithelium (FAE) sample foreign material and deliver it to the organized mucosal lymphoid tissue. The purpose is to initiate the specific immune response. While induction of the immune response occurs in M cells of the Peyer's patches, the effector region is the lamina propria.

Follicular dendritic cells (DCs) are numerous in the subepithelial dome (SED) region located beneath the FAE. DCs are professional antigen-presenting cells (APC) and as such are able to engulf, process, and present the antigen that has been sampled from the intestinal lumen by the M cell. While normally maturing and migrating through the lymphatics or blood vessels to T-cell areas in secondary lymphoid organs, the DCs in the SED region are in close proximity to T- and B-cell zones and thus present antigen locally.

Peyer's Patches: Lymphoid Cells

Lymphoid cells include dendritic cells, macrophages, mast cells, polymorphonuclear (PMNs) leukocytes, natural killer (NK) cells, T lymphocytes (CD4⁺ helper T lymphocytes and CD8⁺ cytotoxic T lymphocytes), and B lymphocytes. Greater than 80% of the body's activated B cells are located in the intestines and 80%–90% of the terminally differentiated B cells are found in the lamina propria IgA blasts and plasma cells (Brandtzaeg et al., 2001; Campbell et al., 2003). This area, the lymphoid cells of the Peyer's patches, is referred to as an inductive site where antigen-activated B cells undergo class switching in order to synthesize IgA. This class switch is linked to the effector site where class switching occurs as a result of cytokines produced by activated T cells and other cells in the intestinal environment. IgA⁺ B cells perform an important function in host defense.

BARRIER DYSFUNCTION

The mucosal epithelium performs numerous specialized functions, discussed above, that are required for health, nutrition, and protection of the body from the outside environment. However, perturbation of this finely balanced homeostasis may result in altered function, such as altered barrier function. Furthermore, the very

immunological mechanisms designed to prevent infection with enteric pathogens may be circumvented by the M cells to actually transport pathogenic microorganisms into the body.

Immune-Induced Barrier Dysfunction

Immunological defense against infection results in the production of various cytokines and chemokines, each with a multitude of autocrine and paracrine effects. While immunological defense is the designated and desired role of the mucosal immune system, infectious disease may regulate in a positive or negative fashion the delicate and tightly controlled homeostatic balance required in the GI tract. Infection by microorganisms results in the production of cytokines by T helper or CD4⁺ cell that is termed either a TH₁ or TH₂ response. TH₁ cells produce IFN γ and IL-2, are active against intracellular bacteria and viruses, and induce isotype switching in humans to IgG1 and IgG3. TH₂ cells produce IL-4, IL-5, and IL-10, are active against extracellular bacteria and parasites, and induce isotype switching to IgA, IgE, and IgG4. Lymphocytes in the intestines include (a) CD8⁺ intraepithelial lymphocytes (IEL) or (b) a submucosal population of CD4⁺ lamina propria lymphocytes (LPL). While CD4⁺ TH₂ T cells are known to produce IL-4, evidence now demonstrates that subsets of IEL also produce IL-4 (Fujihashi et al., 1993a, 1993b). Thus, infection by intracellular bacteria or viruses results in the production of IFN γ while infection by extracellular bacteria or parasites results in the production of IL-4.

Thus, either IL-4 or IFN γ can act directly on epithelial cells to alter the barrier function of the mucosal epithelium and modulate the effect of electrolyte and water loss through the intestinal barrier as a result of a derangement or perturbation of epithelial tight junctions (Watson et al., 2004).

Trojan Horse Barrier Dysfunction: Reovirus, Local, and Systemic Infection through the Peyer's Patches Epithelium

Reovirus (respiratory, enteric, orphan viruses) have been studied in the respiratory and GI tract. Reovirus serotype 1 (Lang) strain and reovirus serotype 3 (Dearing) have been most thoroughly characterized. Reovirus type 1 (Lang) replicates to high titers after oral infection in suckling mice while reovirus type 3 (Dearing) does not. The molecular basis for this differential replication and subsequent viral shedding from the GI tract resides in the L2 and S1 genes. The S1 gene which codes for the outer capsid protein sigma-1 and the L2 gene which codes for the lambda-2 core spike protein are responsible for the difference in allowing the Lang strain, but not the Dearing strain, to replicate in intestinal tissue (Keroack and Fields, 1986; Bodkin and Fields, 1989).

Reoviruses enter the body through the very mechanism that protects and initiates an immune response against infections agents—demonstrating the imperfect barrier function of Peyer's patches. Reoviruses (respiratory, enteric, orphan viruses) were named by Sabin (1959) because they were typically isolated from the respiratory and GI tracts and were not associated with any known disease. Infection in the GI tract occurs using the intestinal M cells as a pathway or portal of entry. Studies in mice

demonstrated that reovirus type 1 was detected adhering to the surface of intestinal M cells, but not other epithelial cells, 30 minutes after administration into the intestinal lumen. Viruses were detected in the M cell cytoplasm within 1 hour (Wolf et al., 1981). Thus, reoviruses attach to the surface of specialized epithelial cells, M cells, that overlie the dome of Peyer's patches. M cells transport macromolecules from the intestinal lumen to the intercellular space. Following facilitated access through M cells, reovirus is spread and is sequentially detected in Peyer's patches, mesenteric lymph nodes, spleen, and the CNS (Kauffman et al., 1983).

Numerous bacteria, viruses, protozoa, and helminths are capable of causing intestinal infections with clinical illness (Table 9.1). The majority of these infectious agents cause diseases that range from mild to severe and include diarrheal illness, dysenteries, enteric fevers, and malabsorptive states. Virulence properties typically include cytotoxic enterotoxins, fimbrial attachment factors, expression of proteins leading to effacement of outer membrane proteins allowing bacteria to be internalized by epithelial cells, cytotoxins that suppress protein synthesis leading to cell death, expression of proteins allowing survival within macrophage phagolysosomes. Two recurrent bacterial properties of enteric bacterial pathogens include: (1) mechanisms allowing adherence to mucosal epithelial cells and (2) elaboration of toxins (reviewed by Levine and Nataro, 1994).

Cholera is an example where the cholera toxin is capable of killing epithelial cells, so that cell to cell junctions no longer maintain the integrity of the fluid barrier between lumen and lamina propria. Body fluid thus accumulates in the lumen and passes out through the intestine as a watery diarrhea, leading to rapid, dehydration and may even lead to death. Treatment with antibiotics and providing hydration therapy either with an intravenous plasma substitute or with a properly balanced solution of salt delivered orally. The cholera bacteria will clear and the epithelium will be replaced by stem cells dividing in the crypts. Enterotoxigenic *Escherichia coli*, enteropathogenic *Escherichia coli*, *Shigella*, rotaviruses, *Vibrio cholera* O1 and *Shigella dysenteriae* 1, salmonella typhi, *Campylobacter jejuni*, enteric adenoviruses and *Entamoeba histolytica* are among the most important pathogens from a public health point of view that cause intestinal disease (reviewed by Levine and Nataro, 1994).

EFFECT OF THE MICROBIOME

The gut contains 1,000 to 1,500 different bacterial species; however, an individual contains only approximately 160 different species indicating that the makeup of an individual microbiome is quite different between individuals and may be influenced by genetics, diet, lifestyle, age, inflammation, and many other factors (Lee and Mazmanian, 2016; Harmsen and Goffau, 2016). This is emphasized by studies by Hasegawa and Inohara (2014) indicating that mice with the same genotype housed in separate cages within the same facility have different microbiota compositions (Shi et al., 2017). The gut microbiome has been reported to influence susceptibility and severity of a number of diseases (Dietert, 2016). Gnotobiotic mice that harbor known bacterial species and germ-free mice with no bacteria are useful models in studies that attempt to dissect and better understand the effects of the microbiome.

Table 9.1 Infectious Microorganisms, M Cells, and the Gastrointestinal Tract

Pathogenic Microorganisms	References
Virus	
<i>Adenovirus</i>	Buller and Moxley (1988)
<i>Astrovirus</i>	Woode et al. (1984)
<i>Breda virus</i>	Woode et al. (1984)
<i>Coxsackievirus</i>	Heinz and Cliver (1988)
<i>Human immunodeficiency virus Type 1 (HIV-1)</i>	Amerongen et al. (1991) and Fotopoulos et al. (2002)
<i>Mouse mammary tumor virus</i>	Neutra and Kraehenbuhl (1992)
<i>Poliovirus Type 1</i>	Ouzilou et al. (2002)
<i>Reovirus Type 1</i>	London et al. (1987), Wolf et al. (1987), Bass et al. (1988), and Helander et al. (2003)
<i>Reovirus Type 3</i>	Wolf et al. (1983)
<i>Rotovirus</i>	Buller and Moxley (1988)
<i>Transmissible gastroenteritis virus</i>	Chu et al. (1982)
Bacteria	
<i>Bacillus Calmette-Guerin (BCG)</i>	Van der Brugge-Gamelkoorn et al. (1985) and Fujimura (1986)
<i>Brucella abortus</i>	Ackermann et al. (1988)
<i>Campylobacter jejuni</i>	Walker et al. (1988)
<i>Chlamydia</i>	Landsverk (1981)
<i>Escherichia coli RDEC-1 strain</i>	Inman and Cantey (1983) and Inman et al. (1986)
<i>Escherichia coli O:124 K:72</i>	Uchida (1987)
<i>Listeria monocytogenes</i>	Jenson et al. (1998)
<i>Mycobacterium paratuberculosis</i>	Momotani et al. (1988)
<i>Rhodococcus equi</i>	Johnson et al. (1983)
<i>Shigella flexneri</i>	Wassef et al. (1989) and Jenson et al. (1998)
<i>Salmonella choleraesuis</i>	Pospischil et al. (1990)
<i>Salmonella enteritidis</i>	Kanoi (1991)
<i>Salmonella gallinarum</i>	Pascopella et al. (1995)
<i>Salmonella typhi</i>	Kohbata et al. (1986) and Pascopella et al. (1995)
<i>Salmonella typhimurium</i>	Hohman et al. (1978) and Jenson et al. (1998)
<i>Streptococcus pyogenes</i>	Nagasaki et al. (1988)
<i>Yersinia enterocolitica</i>	Grutzkau et al. (1990)
<i>Yersinia pseudotuberculosis</i>	Fujimura et al. (1989, 1992) and Clark et al. (1998)
<i>Vibrio cholerae</i>	Owen et al. (1986) and Pierce et al. (1987)
<i>Vibrio parahemolyticus</i>	Yamamoto and Yokota (1989)
Protozoa	
<i>Cryptosporidium</i>	Marcial and Madara (1986)
Prions	
<i>Scrapie Prion Protein PrP</i>	Heggebo et al. (2000)

The gut microbiome has been shown to influence the outcome of cancer immunotherapy in patients by modulating the immune response. Matson et al. (2018) and Gopalakrishnan et al. (2018) compared the gut microbiota from melanoma patients that responded and those not responding to anti-programmed cell death 1 protein (PD-1) immunotherapy and found a correlation between a certain family of bacteria and successful treatment. Routy et al. (2018) found that the gut microbiome influences the efficacy of PD-1 based immunotherapy against epithelial tumors. It is recognized that the microbiome-host symbiotic relationship is an important factor in intestinal homeostasis and mucosal immunity, and it is becoming increasingly important to better understand the influence of the microbiome on immunity and disease.

FUTURE STUDIES

The gut microbiome is a relatively new scientific field of study and has been thoroughly reviewed by Dietert (2016). Additional information will be forthcoming as we learn more about the intestinal cells of the immune system as well as their interaction with other immune cells, with known and yet to be discovered immune cells, immunological mediators, and non-lymphoid cells. These new areas will provide additional information regarding the influence of the microbiome on cells, immunological mediators, disease susceptibility, and therapies for a variety of diseases. One such new approach is the use of mucosal bioengineering or “gut in a dish,” a novel organ culture system that will allow an enhanced understanding of these interactions (Ivanov, 2017). Additional new approaches of *ex vivo* modeling that will further extend knowledge of cell-to-cell and cell-to-immunological mediators include gut-on-a-chip, intestinal organoids, and 2D transwell culture of primary epithelial cells (Kim et al., 2012, 2016; Moore et al., 2014; Clevers, 2016; Ivanov, 2017). Yissachar et al (2017) have designed culture systems that maintained tissue architecture and revealed a role for the nervous system in microbe-immune crosstalk with evaluations of the enteric nervous system pro- or anti-inflammatory responses to microbes. With all of the known interactions of the gut microbiota and disease, the gut microbiome appears to be an additional control center for the immune system.

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CHAPTER 10

Alteration of Intestinal Function by Xenobiotic Exposure

Animal Models

Shayne C. Gad

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The gastrointestinal tract shares with the skin and lung the distinction of being of a major route of both exposure and systemic absorption for environmental chemicals as well as being a target for their adverse (and, in some cases, therapeutic effects). Most pharmaceuticals, food, and water-borne chemicals enter the body via the gastrointestinal tract, whereas occupational exposure to chemicals occurs primarily through skin and lungs. When injury occurs to the gastrointestinal tract by ingestion, deleterious effects frequently effect the whole organism. In addition, damage to the gastrointestinal barrier may enhance or suppress absorption of other substances. Since an intact gastrointestinal tract is necessary for the maintenance of a desired nutritional status, a chemically injured intestinal mucosa (or altered intestinal microbiome) may alter the normal and absorptive functions as reflected by poor systemic nutritional status as well as by altered responsiveness to continued or

additional chemical insults. The response of the gastrointestinal tract to viral agents, microorganisms, or antibiotics may cause a self-limited diarrheal disease. The limited course of such disease tends to remove chemicals via the diarrheal secretions and increased tract motility. Intestinal toxins that result from interactions of chemicals, nutrients, and microorganisms add another dimension to the possible alterations of intestinal function by chemical exposure. As we have become increasingly aware, xenobiotics frequently are systemically toxic by inflammatory mechanisms (Clements, 2009; McKay and MacNaughton, 2012). The possible interactions of chemicals, nutrients, and microorganisms continues to be explored (Nelson, 1976; Tetro and Allen-Vercoe, 2016), with animal gene knockout models being used to evaluate the identities, membrane locations, and metabolic activities of epithelial ion transporters including the secretory isoforms of the Na(+)/H(+) exchanger, and the colonic H(+)-K(+)-ATPase (Shull et al., 2000).

NATURE OF XENOBIOTIC EXPOSURE

The number and amounts of potentially toxic chemicals entering the environment have increased steadily since the beginning of the Industrial Revolution. Their effects went unnoticed or were of little concern initially; however, gradual awareness has developed as to the risk posed to human health by chemically contaminated food and water (Lefebvre et al., 2015). Although the rate of increase in chemical pollution has slowed, thousands of chemicals are still being emitted into the air, dumped into rivers and lakes, buried in the land. It appears that large-scale degradation of surface waters has been stopped; however, water quality data indicate that surface water pollution from conventional and toxic pollutants is still widespread. Groundwater has always been considered a pristine resource, but recent information reveals that in many locations groundwater is contaminated. This contamination comes from many different sources and includes a variety of materials, e.g., synthetic organic chemicals and inorganic chemicals. The concentrations of these synthetic organic chemicals in the ground water are often orders of magnitude higher than those concentrations found in raw or surface water supplies. A third of all public supplies and 95% of all rural domestic depend on ground water resources (El-Hawaii and Plaa, 1978). Based on total use (withdrawal) fresh groundwater is used predominantly for agricultural purposes. The identity of potential pollutants is largely unknown and uncharacterized at present. Comprehensive surveys reveal a vast array of chemical entities encompassed by the compounds added to foods by man. These entities include food additives and residues as well as those occurring as natural toxicants and contaminants. The food additives and residues from migration of packaging materials to food. Estimates of the number of compounds included in the food additive and residue category vary, but the figure of 10,000 is probably conservative. Since 95% of the food reaching the consumer has been processed in some way, the intentional food additives consumed have been estimated as 1.5 kg/yr/capita. As a toxicological consideration, the extensiveness of this consumption makes it difficult to draw attention to individual substances or groups of related compounds.

NATURE OF INTESTINAL FUNCTION

The principal function of the gastrointestinal tract is to modify ingested food so that the nutrients can be absorbed by the intestines, passed into the blood-stream/lymph, and transported throughout the organism. This function involves the processes of secretion, digestion, absorption, metabolism, and motility all working together (Table 10.1). In addition, the intestine serves as a selective barrier to foreign molecules, some of which provoke an antigenic response. Food-and water-borne contaminants are effectively screened.

Chemicals entering the gastrointestinal tract can injure the tract and alter these normal functions as well as modulate their own effects. Interactions of these chemicals within the gastrointestinal tract reflect the properties of the tract per se, the chemicals, and the organisms. Examination of the roles of the gastrointestinal tract in this interaction requires an understanding of the luminal milieu (bacterial and biochemical components) as well as of dietary factors, the brush border, and the intracellular processes (metabolism of nutrients and chemicals). The ingested chemicals may vary in solubility, size, and reactivity, all or which affect their digestion, absorption, and interaction with the mucosal surface. Specific organism factors, such as age, genetic defects, and disease state, also influence the interactions of chemicals with the gastrointestinal tract. The major diseases of the gastrointestinal tract are at least, in part, environmentally determined (Schedl, 1977).

Examination of the effects of ingested environmental chemicals on the gastrointestinal tract as a target organ system may focus on the time-course of damage, recovery, repair, and adaptation within this system. To better understand the impact of these chemicals, model systems are designed to evaluate these effects. This effort develops and exploits animal analogs of the disease processes. A number of approaches have been developed to examine various aspects of gastrointestinal tract function. The choice of approach reflects the focus of the project, which may include enzymes, isolated cells, intestinal sacs, or whole animals. Recent work examines intraluminal metabolism as well as intracellular metabolism.

Approaches to Study Chemical Alteration of Intestinal Function

There is a rapidly growing body of knowledge concerning the effects of ingested chemicals on gastrointestinal function. The approaches utilized in the examination of chemically altered gastrointestinal function include *in vivo* exposure and *in vivo, in situ*, or *in vitro* evaluation, or *in vitro* exposure and *in vitro*

Table 10.1 Major Gastrointestinal Functions

Secretion:	Release of ions, substances, and enzymes
Digestion:	Intraluminal breakdown of ingested substances
Absorption:	Uptake of molecules across mucosal surface
Metabolism:	Intracellular synthesis
Motility:	Movement of intraluminal contents along tract

Table 10.2 Evaluation of Altered Gastrointestinal Function

Method of Exposure/ Method of Monitoring	Function Studied	Environmental Agent	Reference
<i>In vivo/in vivo</i>	Digestion, flora Barrier	Methyl mercury Cadmium	Seko et al. (1981, 1982); Joffe (1971)
<i>In vivo/in situ</i>	Absorption Absorption	Organochlorine compounds Cadmium	Turner and Shanks (1980); Valberg et al. (1977)
<i>In vivo/in vitro</i>	Absorption Motility Absorption	Dieldrin Hexachlorobenzene Lindane; DDT	Kunihara and Meshi (1981); Richter and Schafer (1981); Langman and Bell (1982); Munro (1966)
<i>In vitro/in vitro</i>	Metabolism Absorption Motility	Salicylate Aluminum; DDT and DDE Amitraz (pesticide)	Tanaka and Fromm (1983); Ershoff (1976); Gonalons et al. (1982); Pass and Seawright (1982)

evaluation (Schiller, 1979; Schiller et al., 1983). The various methodologies for evaluating gastrointestinal function were reviewed previously. Specific examples of these approaches taken from the current literature are given in [Table 10.2](#).

Gastrointestinal Functions Affected by Xenobiotic Exposure

Ingested food is processed by the gastrointestinal tract by mixing with a number of secretions produced primarily by the salivary glands, stomach, pancreas, liver, and intestines. These secretions consist of ions, water, enzymes, and bile. In some instances, the degradation processes are assisted by the intestinal flora. Although the salivary glands and their secretions are not essential to life, the secreted electrolytes, K^+ and HCO_3^- , and enzymes, α -amylase, lysozyme, and kallikrein, enhance the digestive process. The stomach elaborates electrolytes, intrinsic factor, pepsinogen, and mucus, which convert the ingested food into semiliquid form.

More specific enzymes enter the intestinal lumen from the pancreas, e.g., trypsin, chymotrypsin, carboxypeptidase, and pancreatic lipase. The liver secretes bile, up to a liter per day in man, which contains bile salts, mucin, hemoglobin breakdown products, phospholipids, cholesterol, and electrolytes. The bile salts are essential for the emulsification of the oil and water portions of the semidigested food and for the formation of micelles. The intestinal secretions play an important role as a lubricant, e.g., mucus.

Examination of the effects of known food and water contaminants and food additives on gastrointestinal secretion/digestion of food is in its infancy. As would be expected, agents that are identified as liver, pancreatic, stomach, or colon carcinogens would be anticipated to affect these normal functions. Also, agents that are destructive of the mucosa surfaces on contact would be expected to disrupt the secretory and digestive functions. Examples of such agents include gastric juices, aspirin (Liboshi et al., 1997), alcohol (Keino and Aoki, 1981), and T-2 toxin (Jacobson, 2017).

Table 10.3 Excretion of Several Chemicals in Feces of Conventional (Control), Germ-Free, or Antibiotic-Treated Animals

Chemical	Conventional	Germ-Free (% of Dose)	Antibiotic-treated	Reference
Warfarin	24	31	33	Rommel et al. (1981)
Inorganic mercury	9.3	—	1.8	Seko et al. (1981)
Diphenylhydantoin	78	—	95	Duggan et al. (1975)

The gastrointestinal tract contains a bacterial system within the lumen, particularly in the colon. These microorganisms contain enzymes that hydrolyze undigested food as well as chemicals. A well-known role of the gastrointestinal flora is the hydrolysis of glucuronide conjugates. Studies have shown that a variety of compounds secreted in the bile as glucuronide conjugates are hydrolyzed by the bacterial 0-glucuronidase, which allows for reabsorption of the compounds. Facilitation of enterohepatic circulation by rat intestinal bacteria has been reported for phenytoin (Duggan et al., 1975), phenacetin (Smith and Griffiths, 1976), diethylstilbestrol (Feinroth et al., 1982), digitoxin (Volp and Lage, 1978), and warfarin (Rommel et al., 1981). More recently, these microorganisms have been demonstrated to convert methyl mercury to inorganic mercury (Seko et al., 1981, 1982; Mahmood et al., 1981; Nedkova-Bratanova et al., 1979). There are many recent examples of the effect of microorganisms on the concentration of ingested substances in the feces (Table 10.3).

The monitoring of substances in the feces is one approach to measuring the relative absorption of agents such as aliphatic hydrocarbons (Albro and Fishbein, 1970). The effects of a dioxin on lipid absorption have been examined in detail with the rat (Schiller, 1982). Prior oral exposure to low doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin markedly augments the appearance of lipid in the serum after a dose of corn oil (Schiller et al., 1982). Several agents have been used to limit the absorption of lipophilic toxins, e.g., cholestyramine (EPA, 1970) and paraffin (Richter and Schafer, 1981). Monitoring radiolabeled carbon dioxide production from radiolabeled nutrients has been utilized to examine malabsorption induced by chemical exposure. In this instance, careful selection of dose and time of monitoring would be essential to detect altered nutrient absorption and subsequent metabolism. Several investigators have used the everted sacs technique for examining alterations in intestinal absorptions of nutrients, e.g., simple sugars and amino acids (Table 10.4).

One aspect of the target organ approach that has changed in emphasis is the recognition that metabolism by an organ is not necessarily synonymous with detoxification. The role of intestinal metabolism of foreign substances by laboratory animals and humans is discussed fully in later chapters. The intestinal mucosa is an extremely active metabolic tissue. The proliferative nature of the crypt cells and differentiation to well-defined absorptive villous tip cells require marked synthesis of macromolecules. For instance, it has been estimated that 50 g of protein is sloughed per day (Westfall and Westfall, 2011). The active transport and synthetic components of intestinal metabolism entail the production of large amounts of metabolic energy. Intestinal absorptive cells contain increased numbers of apical mitochondria. Isolated mucosal cells have been utilized to monitor the unique energy requirements,

Table 10.4 Altered Absorption of Nutrients by Chemicals

Chemical	Species	Method	Effect	Mechanism (Suggested)	Reference
DDT	Rats	Everted sacs	Inhibition of active transport of glucose and tyrosine	Inhibition of sodium pump	Albro and Fishbein, (1970); Dobbins (1982)
Dieldrin	Monkeys	Everted sacs	Augment glucose and depressed leucine uptake	Increased disaccharidase activity	Lefebvre et al. (2015); Chowdhury et al. (1980)
Malathion	Rats	Tissue accumulation	Reduced glucose ^a and glycine absorption	Depressed brush-border enzyme-activities	Fichelle (1971)
TCDD	Rats	Everted sacs	No physiologically significant changes in glucose or leucine active transport	Monosaccharide and amino acid active transport unaffected	Carson, (1962); El-Hawaii and Plaa (1978)

e.g., glucose and glutamine, for this tissue (Shirkey and Schiller, 1980; Schiller et al., 1981). Salicylate alters oxidative phosphorylation monitored in isolated gastric mucosal cells (Tanaka and Fromm, 1983). It has been demonstrated that arsenate inhibits mitochondrial oxidative phosphorylation that is pyruvate-mediated (Flock and Hentges, 1974). Intestinal pyruvate dehydrogenase has been implicated in that inhibitory process (Schiller et al., 1978). The full impact of foreign substances on gastrointestinal metabolism is largely unknown.

Control of intestinal motility is complex and involves both cholinergic and adrenergic nervous system components (Mahmood et al., 1978). In general, cholinergic stimulation increases intestinal motility and adrenergic stimulation inhibits motility. Somnolence and constipation are side effects of many antihistamines (Roberts and Seawright, 1979). A formarnidine pesticide, Amitraz, is widely used to control ticks but with some toxicity to horses (Roberts and Seawright, 1979). The effects of Amitraz on drug-induced contractions of guinea pig ileum *in vitro* indicate inhibition of the histamine H₁ agonists stimulated contractions (Pass and Seawright, 1982). This action may be relevant to the intestinal stasis observed in horses. Recent approaches to monitor motility rely on marker substances other than polyethylene glycol, chromium sesquioxide, and barium sulfate. Of particular value as marker substances are the polystyrene particles, which are available in varying sizes (50–100 μ and 800–1,000 μ) and exhibit low specific gravity (Iturri and Wolff, 1982). Phenol red alone and complexed with a high molecular weight anion exchange resin have also been confirmed as appropriate markers for gastrointestinal transit time in mammals (Katz et al., 1976) and poultry (Fowler et al., 1979).

The second *in vitro* model, the Parallel Artificial Membrane Permeability Assay (PAMPA) (Avdeef and Tsinman, 2006), is the more recently developed. The CACO models (Artursson and Borchardt, 1997; Borchardt et al., 1996) have several caveats associated with their use (e.g., poor predictability for transporter-mediated and paracellularly absorbed compounds, significant nonspecific binding to cells/devices

leading to poor recovery, variability associated with experimental factors) and these need to be considered carefully to realize their full potential.

The Parallel Artificial Membrane Permeability Assay (PAMPA) was first introduced in 1998 (Kansy et al., 1998) and since then numerous reports have been published illustrating the general applicability of this model as a high throughput permeability screening tool (Joffe, 1971; Jilge, 1982; Spidogel et al., 2011; Jacobson, 2017). The model consists of a hydrophobic filter material coated with a mixture of lecithin/phospholipids dissolved in an inert organic solvent such as dodecane creating an artificial lipid membrane barrier that mimics the intestinal epithelium. The rate of permeation across the membrane barrier was shown to correlate well with the extent of drug absorption in humans. The use of 96-well microtiter plates coupled with rapid analysis using a spectrophotometric plate reader makes this system a very attractive model for screening a large number of compounds and libraries. PAMPA is much less labor intensive than cell culture methods, but it appears to show similar predictability. One of the main limitations of this model is that PAMPA underestimates the absorption of compounds that are actively absorbed via drug transporters. Despite the limitation, PAMPA may serve as an invaluable primary permeability screen during early drug discovery process because of its high throughput capability.

PAMPA is a high throughput, non-cell-based permeability model that provides estimates of the passive transcellular permeability property. The lack of any functional drug transporters and paracellular pores in PAMPA makes it an inappropriate model for compounds that are absorbed via transporter-and-pore-mediated processes. However, the lack of transporter- and pore-mediated permeability might be an advantage of the PAMPA model. Because the PAMPA provides uncontaminated transcellular passive permeability data, it could be more useful in constructing the structure-permeability relationship at the chemistry bench. Lipophilicity (most commonly expressed as Log P or Log D values) plays a major role in passive diffusion. An adequate lipophilicity is required for a permanent to travel across phospholipid membrane. However, the PAMPA permeability of 22 marketed drugs did not correlate well with lipophilicity alone because other factors (e.g., polar surface area, molecular volume/flexibility, hydrogen bonding) are also involved in passive diffusion. Although pharmaceutically important drug transporters (e.g., PEPT 1, OCT, OAT) are functionally expressed in Caco-2 cells, they are quantitatively unexpressed when compared with *in vivo* situation. For example, beta-lactam antibiotics (e.g., cephalexin, amoxicillin) and ACE inhibitors, which are known substrates of dipeptide transporters, are poorly permeable across the Caco-2 cell monolayer despite the fact that they are completely absorbed *in vivo*. This model is likely to generate false negatives with drug candidates that are transported by carrier-mediated process. Caco-2 cells have tight junctions that are significantly tighter compared with human intestine, and thus Caco-2 cells normally under-predict the permeability value of drugs that are absorbed primarily via paracellular pathway. The low molecular weight hydrophilic compounds e.g., metformin, ranitidine, atenolol, furosemide, hydrochlorothiazide) showed poor permeability (i.e., equal or less than mannitol) in Caco-2 cells despite adequate absorption (greater than 50% of dose) in humans (Gao et al., 2000). Therefore,

models such as PAMPA and Caco-2 cells can only serve as a one-way screen such that compounds with high permeability in these models are typically well absorbed; however, compounds with low permeability cannot be ruled out as poorly absorbed compounds in humans.

Two *in vitro* models of intentional development have been developed and in variations forms are widely used predictively; these are the Caco and PAMPA models. The Caco system is a cell culture-based assay of the intestinal mucosa using human colon carcinoma cell line (Caco-2) was developed (Hidalgo et al., 1989), which is now widely used by pharmaceutical scientists in academia and industry (Hillgren et al., 1995; Artursson et al., 1996; Bailey et al., 1996; Brayden, 1997; Burton et al., 1997; Gan and Thakker, 1997). The popularity of the Caco-2 cell culture model in studies of intestinal drug transport is due mainly to the ease with which new information is generated. Transport studies using this cell culture model can be performed in large numbers and under controlled conditions, resulting in the generation of a wealth of information. For example, much of the recent knowledge about transporters in the intestinal mucosa has been derived from *in vitro* cell culture models like the Caco-2 cell culture system. The good correlation between passive compound permeation across Caco-2 cell monolayers and that found *in vivo* (Artursson et al., 1996) has made it possible to use this cell culture system (in some situations) to establish structure-transport relationships for drug candidates and to predict intestinal mucosal permeation in humans (Artursson et al., 1996; Lennernas et al., 1996). In fact, the U.S. Food and Drug Administration (FDA) is currently evaluating the application of cell culture assays like the Caco-2 cell system in its biopharmaceutical classification systems for drugs (see internet resources).

To facilitate the use of Caco-2 cell experiments to estimate intestinal permeation of lead compounds, many pharmaceutical companies now perform these experiments in an HTS format. Conversion to an HTS format was made possible by technological advances including: (1) miniaturization of the cell culture apparatus (use of 24-well inserts instead of 6-well inserts); (2) automation of cell culturing and of the actual transport experiments; (3) coupling of cell culture experiments to sophisticated and sensitive analytical methodologies (e.g., liquid sensitive analytical methodologies (e.g., liquid chromatography/tandem mass spectrometry; LC-MS/M); (4) development of standardized methods for quantitation of transport data; and (5) development of sophisticated data systems for the analysis, storage and retrieval of transport data.

In addition to using this cell culture assay to estimate the intestinal mucosal permeation of compounds, the Caco-2 cell culture system has also been used in many mechanistic studies of compound transport and delivery (see [Table 10.5](#) for a summary of the applications of the Caco-2 cell culture system). The development of cell culture assays represents one of the most exciting advances in the pharmaceutical sciences. These types of systems, if properly used, can not only lead to an improved understanding of the biochemical basis of the barrier properties of the intestinal mucosa, but can also potentially expedite the process of drug discovery and development, and thus improve the efficiency of the drug discovery/development process in the pharmaceutical industry. However, in order to maximize the benefit from these technologies, meaningful refinements must be made, such as inducing

Table 10.5 Application of Caco-2 Cell Culture System to Mechanistic Studies of Drug Transport

Applications	References
Elucidate pathways of drug transport (e.g., paracellular versus transcellular; passive versus carrier-mediated) across intestinal mucosa	Knipp et al. (1997)
Determine optimal physiochemical characteristics (e.g., hydrogen bonding potential, conformation) of a drug for passive diffusion via the paracellular or transcellular pathways across intestinal mucosa	Burton et al. (1996)
Determine structure-transport relationships for carrier-mediated pathways (e.g., peptide transporter of drug transport)	Hidalgo and Li (1996)
Determine structure-transport relationships for apically polarized efflux systems (e.g., P-glycoprotein) in intestinal mucosa	Burton et al. (1997)
Determine how components of a formulation (e.g., adjuvants) might influence intestinal mucosal transport of drug candidates	Nerurkar et al. (1996)
Assess potential toxic effects of drug candidates or formulation components on intestinal mucosa	Tang et al. (1993)
Elucidate potential pathways of drug metabolism in intestinal mucosa	Schmiedlin-Ren et al. (1997)
Determine potential drug-drug interactions during intestinal mucosal transport	Wacher et al. (1996)

the expression of underexpressed transporters and enzymes to *in vivo* levels by cell biology methods, shortening culturing time, miniaturizing the cell culture apparatus and automating the transport and metabolism experiments (Artursson and Karlsson, 1991; Borchardt, 1995).

Caco-2 cells, when grown to polycarbonate filters, form confluent monolayers that can be used as an *in vitro* model of the intestinal mucosa. By measuring the permeability of a compound across these cell monolayers, one can estimate the extent of its permeation through the intestinal mucosa.

INTESTINAL TRANSIT

Drugs which modify intestinal transit include atropine, morphine, clonidine, papaverine, and isoproterenol. Besides this *in vivo* test, a number of *in vitro* techniques have been developed to evaluate the effects of test compounds on visceral smooth muscle function. Among them, a method for studying peristalsis (Van Nueten et al., 1973) is particularly valuable in producing results predictive of activity on intestinal transit. However, this test does not meet the requirements of safety pharmacological drug testing which is intended to assess the possible direct/indirect effects of drugs. For these studies, an *in vivo* assay is preferable. The intestinal transit assay presented in this unit (see Basic Protocol 1) is very simple, reliable, reproducible, and relatively low in cost with no requirement for expensive equipment. The assay is widely used as a primary screening assay and as a test for safety pharmacology (LaCroix and Guillaume, 1998).

Table 10.6 Treatment Groups for Measurement of Intestinal Transit

Group	Treatment
1	Vehicle control
2	Test compound, dose 1
3	Test compound, dose 2
4	Test compound, dose 3
5	Reference compound (positive control) ^a

^a For measurement of intestinal transit, the reference compounds are atropine sulfate at 0.8 mg/mL and morphine at 4.8 mg/mL.

Table 10.7 Effects of a Test Compound on Intestinal Transit in the Rat

Treatment ^a	Dose	Distance (cm ± SEM) Covered by Charcoal	Percent transit (±SEM) ^b
Control	0.5% CMC ^c	80 ± 5	70 ± 6
Atropine sulfate	2 mg/kg	54 ± 6	50 ± 2 ^b
Morphine hydrochloride	12 mg/kg	51 ± 3	50 ± 3 ^b
Test compound	10 mg/kg	68 ± 4	62 ± 3
Test compound	30 mg/kg	66 ± 6	60 ± 4
Test compound	100 mg/kg	72 ± 3	70 ± 3

- ^a Single oral dose of vehicle, reference compound (atropine sulfate and morphine hydrochloride), or test compound (glucose) was administered 1 hr prior to the administration of charcoal suspension. Values are mean ± standard error of the mean (SEM), with eight animals per group.
- ^b $P \leq 0.001$ for intergroup versus control comparisons.
- ^c Control is 0.5% carboxymethylcellulose.

The effects of drugs on the extent of intestinal transit during a fixed time period following oral administration of a test compound can be evaluated in the rat. The measured endpoint is the extent of passage of a charcoal suspension through the small intestine (Macht and Barba-Gose, 1931; Fischer et al., 1973). Atrophine sulphate and morphine hydrochloride (reference compounds) are used as a positive control. For each test compound, five groups of eight animals each are used (Table 10.6). This protocol can also be used in the mouse to measure intestinal transit, wit the modifications noted.

Typical results are shown in Table 10.7.

Inflammatory Bowel Disease (IBD)

Inflammatory Bowel Disease (IBD) is a group of chronic inflammatory intestinal conditions (such as Crohn’s disease and ulcerative colitis) which has become more prevalent in first world populations and which have been associated with a range of drugs (such as Accutane). These inflammatory conditions may affect any region of the GI tract, are extremely disabling, and can be either due to systemic immune

modulated conditions or direct chemically induced innate immune response of the tissue (though these later are usually resolved in the short-term).

Goyal et al (2014) present a useful overview of animal models of these diseases for use in exploring treatment modalities. Mice, rats, and dogs are the most common model species (Jiminez et al., 2015).

Ulcerogenic Activity

Mucus acts as a lubricant barrier to protect the surface of the gastrointestinal tract against acid, pepsin, and the mechanical forces of digestion (Flemström and Garner, 1982; Hiam et al., 2012). Non-steroidal anti-inflammatory drugs (NSAIDs) decrease the thickness of the mucus gel layer (Menguy and Masters, 1965) provoking mucosal erosion and ultimately ulcers. Similarly, a serious side effect of adrenocorticotrophic hormone (ACTH) and corticoid therapy in man is development or reactivation of gastroduodenal ulcers (Robert and Nezamis, 1958). A variety of other agents, including ethanol (McQueen et al., 1983), also cause damage to the gastrointestinal tract (Lwoff, 1971). The ulcerogenic assay described in this unit (see Basic Protocol 2) is very simple, reliable, and reproducible. It is used widely for safety pharmacology.

Estimation of luminal mucus in gastric washings has been used as an index of secretion (Bolton et al., 1978; Johansson and Kollberg, 1979). Among the techniques developed for measuring the dimensions of the gastric mucus layer is a slit lamp and pachymeter (Bickel and Kauffman, 1981) and a direct microscopic method (McQueen et al., 1983). Basic protocol 2 is a rapid assay that allows easy detection and quantification of the ulcerogenic potential of test substances however, this assay provides no information on the effect of test substances on the gastric mucus layer. The ulcerogenic effects of drugs can readily be evaluated in the rat. In this assay, test compounds are administered orally to rats once daily for 1–4 days, then the stomach and the duodenum are removed and scored for irritation and ulcers. Indomethacin (reference compound) is used as a positive control. For each test compound, 10 groups of 8 animals each are used—5 groups for examination on day 1, and 5 additional groups for examination on day 4 (Table 10.8).

Table 10.8 Treatment Groups for Measurement of Ulcerogenic Activity

Group	Treatment	Evaluated on Day
1	Vehicle control	1
2	Vehicle control	4
3	Test compound, dose 1	1
4	Test compound, dose 1	4
5	Test compound, dose 2	1
6	Test compound, dose 2	4
7	Test compound, dose 3	1
8	Test compound, dose 3	4

CONCLUSIONS

The GI tract is clearly understood to be a target organ system for ingested substances, with local and systemically mediated effects on itself as well as the full range of systemic targets and toxicities. This appreciation is reflected in the increasing interest in the gastrointestinal tracts a metabolic organ contributing to the homeostasis of the entire organism. The areas of gastrointestinal research currently at the forefront include the role of the bacterial ecosystem (microbiome) in metabolism of nutrients and foreign substances (Fischer et al., 1973; Savage, 1981) and the role of enterohepatic circulation in affecting the absorption of substances from the lumen (Chowdhury et al., 1980; Spidogel et al., 2011), the role of fibers in digestion, absorption, and protection of the intestines (Story, 1981; Kapp, 2014), and response of the local gastrointestinal immune system to toxic substances (Colburn, 1982; Spidogel et al., 2011). Adaptation normally occurs in the gastrointestinal tract in response to increasing age, enteral feeding of nutrients, and after surgical removal of a section of intestines (Schiller, 1982). The ability of the gastrointestinal tract to adapt and develop tolerance after toxic insults is extensive and subject to growing understanding. Because of the high metabolic and cell turnover rates of the intestinal mucosa, it is not a surprise that this tissue is particularly susceptible to injury. Only more recently understood are the systemic immune system aspects of these interactions.

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CHAPTER 11

The Role of the Microbiome on Metabolism, Absorption, and Toxicity of Xenobiotics in the Gastrointestinal Tract

Shayne C. Gad

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INTRODUCTION

While there was wide but diffuse recognition that the population of microorganisms in the GI tract of mammals had significant influence on both the health of humans and other higher organisms (predating Lederberg and McCray's, 2001 use of the specific terms "microbiome" and "microbiota"), the influence of such was well recognized in the 1960s (Prescott, 2017)—see the author's 1966 science fair project on the subject (Gad, 1966), and led to the development and use of "germ free" animals in research in those years. However, it only became a significant area of popular focus over the last 30 years. Indeed, it was not addressed in the last edition of this book. The NIH Human Microbiome Project (HMP) in 2009 launched a concerted

effort to characterize and understand this major body component in both animals and humans (NIH HMP Working Group et al., 2009).

While bacterial populations are normally common to a number of regions of the human (and mammalian) bodies (such as the vagina and nasal passages), here we are going to focus on those that are normally resident in the GI tract.

The current estimate is that there are ~30 trillion component cells in the human body, 84% of which are red blood cells (Hewings-Martin, 2017). These cells are extremely varied, with the largest by mass being fat cells, which make up nearly 19% of body mass but less than 0.2% of cell numbers.

The main types of cells in the human body are stem cells, erythrocytes, leukocytes, myocytes, and cartilage cells.

The number of bacteria in our bodies, however, is estimated to be 10 times the number of human cells, though their mass (~200 g) is much less. The variety of cells potentially represented, however, is enormous (1,000–1,500 different species), and the composition of the microbiome, as that of humans, is extremely variable (any one individual has only 160 varieties of bacteria present at any one time in their gut [Li et al., 2012; Lee and Maymanian, 2016]), making studying and understanding both and their interactions difficult at first. The same is true of understanding and generalizing the influences of the microbiome on the toxicology (and pharmacology) of xenobiotics (Li et al., 2012). Studies in humans are either in volunteers (the primary basis of the HMP) or in postmortem studies (Pechal et al., 2018). Human health and that of all mammalian species, is both influenced by and dependent on these bacteria. The exact nature of the constituent biota is a reflection of age, diet, environment, and physiologic (and pathophysiologic) state.

Much of the current focused interest on the microbiome comes from the belief that population and indirectly medicine and health can be positively influenced by altering the microbiome. There are obviously multiple dimensions to this possibility, as the interaction variables at play (interacting) are host (for which there will be variability due to disease stage, age, nutrition, and genetic background), the microbiome itself (for which species, numbers, and environment—location in the host organism) xenobiotics and their metabolism and toxicity (dose, concentration, and purity) and the environment external to the host must all be considered.

For our purposes here, focus will be on the microbiome of the GI tract from the point of view of the general case (normative) condition and the assumption will be that the existent normative microbiome will act to maintain a “normative” condition.

Points to be considered here will be:

- Species differences
- Biome effects on hosts
- Host effects on the microbiome
- Microbiome effects on absorption of xenobiotics (including nutrients)
- Microbiome effects on metabolism of xenobiotics
- Effects on efficacy
- Effects on toxicity
- Cases where the microbiome itself is “toxic” (such as *H. pylori*)
- Influence on and of pathophysiology

Species Differences

As one would expect, there are significant variations in what species constitute the microbiomes of different mammalian species. Though this chapter will focus on the ins and outs of the human case, most of our actual studies are done in other species. It is thus important that we consider how these are different from humans.

Major sources of variation are diet, environment, and genetics (Gad, 2016). Muegge et al (2011) looked across 16 non-laboratory species—all herbivores—and found that dietary similarities tended to cause convergences in the microbiome population. The same is true of laboratory species, but variations in optimal diets causes discrepancies. Diets rich in saturated or polyunsaturated fat (such as fish oil) can be profoundly influenced both by the gut microbiome composition and the immune system of the host (Devkota et al., 2012).

Differences may also be in response to species differences in cytochrome P450 enzymes (CYP) (and other major metabolic enzyme systems) mediated drug metabolism, inhibition, and induction (Martignoni et al., 2006), though it is also important to realize that many species have a commensal relationship with their microbiota, depending on them for primary digestion of foods (such as cellulose and production of micronutrients).

A review of common laboratory animal species (Bleich and Fox, 2015) determined that some 500–1000 species may inhabit their intestines and that these complex populations are in a subtle state of homeostasis unless their environment is altered. These microbiota also serve to protect their hosts against pathogens, however, as in humans, the microbiomes can profoundly alter the invitation and progression of diseases such as metabolic syndrome, obesity, diabetes, inflammatory bowel disease, autoimmune arthritis, and irritable bowel disease. Use of germ-free animals may simplify their use as models for human disease, but also can give misleading answers. Several researchers have started with germ-free animals and repopulated their intestines with human-derived microbiota to allow better assessment of the human condition. However, at least in mice, lack of a microbiota leads to major defects in the development of gut-associated lymphatic tissues (Round and Mazmanian, 2009).

Microbiome Effects on Hosts

Most of us are already aware (at least superficially) of effects that the gut flora can have on our daily lives. Ranging from *H. pylori* being a major cause of duodenal ulcers, to changes in digestion and GI function after a course of antibiotics changes our baseline GI flora (*dysbiosis*), we experience excursions from normal interaction with our gut flora on a regular basis. These organisms assist in the conversion of nutrients and detoxification, in protecting against microbial and other pathogens (competitors), and in supporting normative immunity in the GI tract (the major portal of external agents into the body) (Scotti et al., 2017; de Zoete et al., 2018). The population is not limited of course, to bacteria, but will also include fungi and viruses.

Bacteria do have defined functions in the human gastrointestinal tract. Limiting ourselves here to GI microbiota, these include (Kinross et al., 2011; Koppel et al., 2017):

- Vitamin synthesis
- Metabolism of carcinogens and toxicants
- Formulation of non-digestible dietary residues
- Fortification of barriers (by biofilm formation)
- Tightening of intractable junctions
- Immune system development and function (Castellino, 2018)
- Producing antimicrobial factors
- Displacement of pathogens
- Metabolic transformation and absorption

They also can interact with the metabolism and absorption of drugs, influencing efficacy and toxicity (Kang et al., 2013; Li et al., 2016; Wilson and Nicholson, 2017; Curro, 2018), as we will look at in more detail later. There are actual functional interactions between diet, microbiota and host organisms (Greiner, 2014; Kong et al., 2014).

Microbiota populations can clearly be causative or contributory to oral disease (cavities and periodontal), metabolic disease (obesity, cardiovascular disease, type 2 diabetes), pathologies of the digestive tract (celiac disease, nonalcoholic hepatitis, and inflammatory bowel disease), colorectal cancer, colorectal adenoma, hepatobiliary cancers, and food allergies. Less prominent but more commonly, the microbiota regularly alter the functionality of the GI tract (Kovatcheva-Datchary et al., 2009).

More profoundly, the microbiota frequently influence the development and progression of inflammatory, adaptive, immune, and autoimmune diseases (Kovatcheva-Datchary et al., 2009; Karczewski et al., 2014). They can act to prevent, limit, or provoke autoimmunity—a disease set the significance of which we are increasingly becoming aware (Parekh et al., 2014; Sathyabama et al., 2014; Ohno, 2015; Scotti et al., 2017).

Host Effects on the Microbiota

The most obvious ways a host can affect their microbiota is by dietary change. Diets low in refined sugar and fats tend to lead to the greater diversity in resident microbiota, which tends to improve stability of the bacterial population (Kong et al., 2014). The interactions change depending on the age, health, nutrition, and (especially) disease status of the host (Kinross et al., 2011).

Smoking, inflammatory diseases, use of probiotics, aging, obesity, and inflammatory diseases (plus, obviously, use of antibiotics) also significantly influence the composition of microbiome populations.

Any actions of the host which alter the environment of the microbiota in one or more regions of the GI tract can alter the homeostatic balance of the resident

community, suppressing and depleting a portion of the normative population by changing the chemical environment (pH osmolarity, antimicrobials, or a change in available nutrients), and thereby making room for opportunistic pathogens such as *Clostridium difficile*, *Salmonella typhimurium*, or *E. coli*, which in turn will act on the host to additionally modify their environment (Lichtman et al., 2016). Physiologic and pathophysiologic changes arising elsewhere in the body, particularly if they evoke or involve an inflammatory or innate immune response in the tissue of the GI tract, will likewise serve to cause population changes in the microbiota. Looking at changes in tissues, the GI tract is typically blind to changes in the microorganisms resident in the tract that were present and involved in the progression (and perhaps recovery) of morphologic changes in the tissue. The prime example would have to be *H. pylori*, an unrecognized major actor in the development of ulcers. *Salmonella* invades intestinal cells by a secretion system, promoting intestinal permeability. *Clostridium*, on the other hand, remains extracellular but secretes toxins that are taken up by intestinal epithelia and induce apoptotic cell death.

Hosts do not have the constant genomic changes that the microbiota do. The proteins expressed by host cells thus are generally much less variable than those of the microbiota. Unlike when the author studied mouse microbiota and the effects of radiation on them in the mid-1960s, presently the tools of proteomics provide a much more sensitive and real-time means of following GI tract changes. The results of interactions and changes can also be evaluated by analysis of stool.

Inflammatory states in hosts have specific signatures, both in the morphology of host tissues in alternation in excreted proteins, and in changes in microbiota populations (Cooper et al., 1993; [Table 11.1](#)).

Effects of Microbiota on Absorption of Xenobiotics

Short-term and long-term, acutely or chronically, the microbiota influence the absorption of xenobiotic compounds (be they nutrient, drugs, or potential toxins). The entire concept of absorption, distribution, metabolism, and excretion (ADME), which is fundamental to toxicology and pharmacology, yet the influence of the microbiome (in the case of the oral route of exposure) has been (and largely still is) ignored.

The all-important initial absorption step is viewed with the assumption that the most important aspects are the chemical and physical characteristics of the xenobiotic itself as they present at the membrane surface of the potential absorptive tissue (Silbergeld, 2017).

Before the molecule ever gets to that surface, it may well be subject to an initial set of metabolic and biologic modifications rising from the host microbiota—particularly as most absorption occurs in the intestines, where the microbial population is richest and most diverse. Additionally, the resident microorganism can modify the surface of the absorptive tissues.

The microbiota acids in the colon (Greiner, 2014) and stimulated secretion of incretin hormone GLP-1, which affects intestinal transit speeds. They also degrade intestinal secreted mucus. At the same time, the short-chain fatty acids (SCFAs) that

Table 11.1 Beneficial Normal Microbiota and Their Subsets in Immune Response

Bacteria/Subset	Immune Response
Segmented filamentous bacterial	Th17 cell induction
Segmented filamentous bacteria	T cell response
<i>Bifidobacterium infantis</i>	Induces FOxP3 T cells through TLR-4 pathway Induction of Treg cells through TLR-4 pathway
<i>Lactobacillus rhamnosus</i>	Novel hyporesponsiveness of T cells in Crohn's patients via production of TGF-β DC maturation and protection in colitis model and high expression of immunoregulatory enzyme indolamine-2,3-dioxygenase
<i>Lactobacillus casei</i>	Induces Tregs via binding toC-type lectin DC specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)
Gut derived lactic acid bacteria	Initiates NK-DC interaction via DC maturation
Short-chain fatty acid	Anti-inflammatory response, enterocyte nutrition
Peptidoglycan of Gram-negative organism	Neutrophil priming
<i>B. fragilis</i>	Induce T cell response with PSAs
Lipoteichoic acids	Induction of TLR 2
Staphylococcal lipoteichoic acids	Induction of TLR-3
DNA of <i>Bacteroides thetaiotaomicron</i>	Enhancement of NFκB and Treg cells through TLR-4 pathway
Lipopolysaccharide	Increase in CD4 ⁺ CD25 ⁺ T cells
<i>Staphylococcus aureus</i>	Produce antimicrobial proteins and produce biofilm

Source: Hooper, L.V. et al., *Science*, 336, 1268–1273, 2012; Belkaid, Y. and Hand, T., *Cell*, 157, 121–141, 2014; Sathiyabama, S. et al., *Crit. Rev. Microbiol.*, 40, 273–280, 2014.

are produced (such as butylate, acetate, lactate, and propionate) serve to lower the luminal pH and nourish colon cells.

Effects of Microbiota on Metabolism

The gut bacteria are extremely important in the metabolism of orally ingested xenobiotics. At one end of the spectrum are their metabolism of nutrients such as taurine and choline, and their essential role in the absorption of fatty acids. Most of the energy for the microbiota to perform this comes from sugars, and many of the fatty acids are essential for the nutrition of the enterocytes of the wall of the intestines. A large number of essential vitamins come from the action of the gut microbiota—biotin, and vitamins A and K.

The physiologic normative (or otherwise) state of the host is dependent on the intestinal (that is, in homeostasis) microbiota due to their multiple influences on the host.

Kang et al. (2013) provide an extensive review of natural products that require metabolism by intestinal bacteria to have their desired pharmacologic effects (Table 11.2).

Table 11.2 Effects of Intestinal Microbiota on Metabolism

Metabolized Xenobiotics	Product Absorbed by Host	Importance
Sulfasalazine	Reductive metabolite	To treat ulcerative colitis in intestines
Prontosil	Sulfanilamide	Antibiotic
Neoprentosil		
Nitrazephan	Reduction to 7-aminonitrapam	Benzodiazapine hypnotic
Tamoxifen	Phase II sulfation	
Apomorphine	Phase II sulfation	
Mercury (HgII and O)	Detoxification and reduced absorption	
Aminosalicylates	Class taken as prodrugs and activate	Anti-inflammatory
Anthranoid laxatives	Products activated by gut flora	
Digoxin	Inactivated by gut bacteria	Cardiac glycoside for cognitive heart failure
Drinotican	Deconjugation by B-glucuronidases	Release of active drug moiety
Balcalin	Hydrolysis follow by metabolism of active component	Activation and metabolism
Acetaminophen	Microbial metabolism influencing both absorption and toxicity	Anagenic and antipyretic

Source: Roberfroid, M.B. et al., *Nutr. Rev.*, 53, 127–130, 1995; Kang, M.J. et al., *Expert Opin. Drug Metab. Toxicol.*, 9, 1295–1308, 2013; Bojic, G. et al., *Zbornik Matice srpske za prirodne nauke*, 47–55, 2015; Lu, K. et al., *ILAR J.*, 56, 218–227, 2015; Curro, D., *Expert Rev. Clin. Pharmacol.*, 11, 171–183, 2018.

Effects of Microbiota on Toxicity of Xenobiotics

Intestinal microbiota can metabolically improve the efficacy of drugs (by activating them from prodrugs and improving their absorption), reduce their efficacy (by deactivating active moieties prior to absorption), or cause drugs—and other xenobiotics—to be toxic, or more toxic. Examples are presented in [Table 11.3](#).

The types of metabolic interactions can be thought of as

1. Direct metabolism of xenobiotics
2. Interference of xenobiotics with normal microbiota metabolism
3. Metabolism of xenobiotics after their conjugation (Phase 2 reactions) by liver
4. Dysbiosis—alteration in microbiota populations by xenobiotics

Microbiota Effects on Drug Effectiveness

Gut bacteria are well established as influencing the efficacy of drugs administered that route, ranging from reducing the efficacy of digestion to enhancing or degrading the efficacy of the immunotherapeutic oncology drugs such as checkpoint inhibitors (NCI, 2018), due to the combined actions of intestinal and bacterial transporters. The intestinal microflora mainly act by reductive and hydrolytic reactions which generated non-polar low molecular weight byproducts (Sousa et al., 2008).

Table 11.3 Toxicity Due to Microbiota Metabolism

Metabolism of	Toxicity	Toxic Metabolite
Chloramphenicol	Bone marrow aplasia in 1% of patients	p-aminophenyl-2-amine-1,3-propamediol
Melamine	<i>Klebsiclla</i> increases the production of cyanuric acid	Causes renal damage, formation of bladder and renal stasis
Metronidazole	Anaerobic bacteria form acetamide and other moieties by ring cleavage	Liver toxicity
Polycyclic aromatic hydrocarbons (PAHs)	C-S-lyase	Methylsulfone metabolites
Digoxin	Inactivated	By <i>Eggothella levta</i>

Source: Weinhold, B., *Environ. Health Perspect.*, 121, A149, 2013; Haiser, H.J. et al., *Gut Microbes*, 5, 233–238, 2014; Stojančević, M.D. et al., *Curr. Issues Mol. Biol.*, 16, 55–68, 2014; Ohno, H., *Semin. Immunopathol.*, 37, 1–3, 2015; Claus, S.P. et al., *Biofilms Microbiomes*, 2, 16003, 2016.

Evaluating the Microbiota and Xenobiotic Interactions with Them

In 1966, the means of studying the microbiota in a species was largely limited to collecting samples from dead animals or humans, collecting intestinal contents, culturing the microbiota and identifying species by means of *Bergey’s Manual of Determinative Bacteriology* (Gad, 1966; Bergey and Holt, 1994). The limits to this approach are obvious—the sample gave a poor representation of the actual microbiota, and one limited to a single issue from a single animal. Plus, of course, the methods were laborious, expensive, and gave crude answers.

Using mucosal biopsies offers only some improvement on sample validity, as does sampling based on fecal microbiota.

Currently, we are seeing capsules that can be programmed to sample specific regions of the GI tract as they transit it. As more data and experience are gained, we should expect significant improvement of methodology.

CONCLUSION

To date, regulatory toxicology ignores the intestinal microbiome in identifying and evaluating adverse effects of drugs and other xenobiotics, with the modest exceptions of noting the physiologic and immunologic signs which may be associated with disruption of what, in effect, is another system involved in the normal and abnormal functioning of a person or of another mammal used as a model for man. Even histopathological techniques are largely designed to exclude the microbiota from the evaluation of toxicity of a xenobiotic. In doing so, a lot of explanatory data is lost. Especially as we become more aware of the broad involvement of inflammatory and other immune-based responses in serous and chronic diseases, it will be essential to capture and incorporate the microbial component of mammalian systems into our assessment of benefits and risk.

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CHAPTER 12

Normal and Abnormal Intestinal Absorption by Humans

David W. Hobson and Valerie L. Hobson Balldin

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The primary function of the gastrointestinal (GI) tract in humans and other animals is to process food materials and absorb nutrients from these materials that are required for cellular metabolism and for maintaining normal systemic functions. The process of digestion and absorption in the GI tract is essential both in terms of maintaining the nutritional status and homeostasis of the entire body. The GI tract is the primary organ system responsive initially to the composition of the diet and mediates as well as modulates variations in dietary consumption and composition. Since the processing and absorption of nutrients within the GI tract is highly dependent on normal intestinal function, it is important to understand the processes that are involved as well as how intestinal absorption affects the human GI response under both normal and abnormal conditions. This understanding is critical for studies intended to show how toxicants and toxins may affect GI function and the overall metabolic response of the whole organism.

The digestive and absorptive functions of the GI system depend on a variety of mechanisms that soften the food, propel it through the GI tract, and mix it with hepatic bile stored in the gallbladder and digestive enzymes secreted by the salivary glands and pancreas. Some of these mechanisms depend on intrinsic properties of the intestinal smooth muscle; others involve the operation of reflexes involving the neurons intrinsic to the gut, reflexes involving the central nervous system (CNS), paracrine effects of chemical messengers, and GI hormones. The hormones are humoral agents secreted by cells in the mucosa and transported in the circulation to influence the functions of the stomach, the intestines, the pancreas, and the gallbladder, and can act in a paracrine fashion.

Some of the principal functions within the GI tract critical to its role in nutrition and human health are shown by principal regions in [Table 12.1](#).

Table 12.1 Regional Functions of the Human Gastrointestinal Tract in the Absorption of Nutrients and Maintenance of Fluid and Electrolyte Balance

Gastrointestinal Location	Normal Absorption Function(s) and pH
Oral Cavity	<i>Mastication, maceration of food, fluid intake, saliva production.</i> pH 6–7
Esophagus	Food and fluid transport to stomach. pH 4–6
Stomach	Digestive processing (acidic and enzymatic) to absorbable units. pH 1–2.5
Pylorus	Muscular control of absorbable unit entry from the stomach into the small intestines
Small Intestine (duodenum)	Mixing with bile and pancreatic secretions; Iron and Calcium absorption. Bicarbonate secretion. pH 4–8
Small Intestine (jejunum)	Intestinal digestion. pH 6.5–8
Small Intestine (ileum)	Absorption of fat-soluble vitamins (B ₁₂ especially), other nutrients, fatty acids and bile salts. pH 7–8
Caecum	Separates intestinal contents from microbial-laden colon contents. pH 5.7–7
Colon (Ascending)	Fluid absorption; some protein absorption; liquid to solid conversion. pH 6–7
Colon (Transverse)	Fluid absorption; liquid to solid stool formation. pH 6–7
Colon (Decending)	Stool formation; fluid absorption. pH 6–7
Colon (Sigmoid)	Stool formation and storage; some fluid absorption. pH 6–7
Rectum	Stool formation and storage; stool retention and expulsion control. pH 6–7
Anus	Stool release; stool retention and expulsion control

The diets of early humans, sometimes termed the “paleodiet,” were extremely varied due to a wide range of plant species and plant organs that were consumed over wide geographic areas [1]. Foods of animal origin included combinations of those taken opportunistically, such as invertebrates, amphibians, reptiles, small mammals, birds and their eggs, and the scavenging and hunting of larger mammals. Each of these types of food has characteristic nutritional compositions. Studies of the compositional features of the diets of early humans and native populations reveal that an adequate diet could be obtained in a variety of ways. The human gut microbiome represents a diverse microbial community that varies across individuals and populations, and diet is a major force shaping the gut microbiome. Diet is also influenced by factors such as host genetics and lifestyle [2]. Obtaining adequate water and energy are likely to have been the main physiological drives and the selection of fat containing foods probably had substantial advantages in reducing the amount of plant foods that needed to be gathered due to their ability to provide essential nutrients as well as a higher degree of satiety. Then, just as now, many plant foods contained, in addition to nutrients, natural toxicants that needed to be recognized and either avoided or eliminated by processing in some fashion or by cooking. Although it is certain that primitive humans must have possessed a vast knowledge of the consequences of consuming acutely toxic plants, it has also been surmised that a preference for sweet tastes could have played a role in protecting early humans from consuming sometimes bitter, toxic plants [1].

Although there are still some primitive populations in existence that consume traditional foodstuffs regularly in the modern world, it is still the case and is well known that the sources and estimated daily amounts of essential nutrients ingested by adults vary worldwide. This is primarily due to differences in dietary preferences and the type and nature of available food stuffs. Current values estimated by the U.S. Department of Agriculture for Americans are shown in [Table 12.2](#) [3]. The recommended daily water consumption is between 2 and 3 L. The data shown in [Table 12.2](#) pertains to dietary solids only and does not include the absorption of water and fluids from endogenous secretions that also are processed and absorbed daily. It has been estimated that endogenous secretions account for about 6–7 L of water overall and an additional 35 to 100 g of protein and between 20 and 30 g of fat [3].

Table 12.2 Estimated Daily Nutrient Intake for Adults^a

Nutrient	Amount
Protein, g	91
Carbohydrate, g	271
Total fat, g	65
Saturated fat, g	17
Monounsaturated fat, g	24
Polyunsaturated fat, g	20
Linoleic acid, g	18
Alpha-linolenic acid, g	1.7
Cholesterol, mg	230
Total dietary fiber, g	31
Potassium, mg	4,044
Sodium, mg	1,779
Calcium, mg	1,316
Magnesium, mg	380
Copper, mg	1.5
Iron, mg	18
Phosphorus, mg	1,740
Zinc, mg	14
Thiamin, mg	2.0
Riboflavin, mg	2.8
Niacin equivalents, mg	22
Vitamin B ₆ , mg	2.4
Vitamin B ₁₂ , µg	8.3
Vitamin C, mg	155
Vitamin E, mg	9.5
Vitamin A, µg	1,052

^a U.S. Department of Agriculture Food Guide nutrient values based on population-weighted averages of typical food choices within each food group or subgroup at the 2000-calorie level.

The GI tract has a very high nutrient demand in large part due to the high turnover of epithelial cells along the villi and the daily synthesis and secretion of protein as a digestive enzyme. Protein turnover in the GI tract is estimated to account for about 20% of total body protein turnover, although the GI tract is typically less than 5% of total tissue mass in the body. The intestinal organs are able to derive part of their nutrient needs from the nutrients present in the lumen during digestion. In fact, the hormones released during the gastric and intestinal phases of digestion to stimulate secretion and motility also have trophic effects which ensure cell and enzyme renewal during the postprandial phase [4].

The luminal presence of nutrients is requisite to maintaining normal GI tract function. Research comparing enteral and parenteral feeding demonstrates that atrophy of intestinal tissue occurs when luminal nutrients are not present to support growth [5]. Because of high nutrient requirements, GI function may be severely compromised during malnutrition. The potential consequences of malnutrition on GI function include:

- Decreased digestion of food materials by pancreatic enzymes
- Mucosal surface atrophy
- Impairment of the immune functions of the GI tract
- Increased microbial activity in the colon due to decreased intestinal motility and the greater presence of undigested substrates

In a malnourished state the GI tract can fail to function effectively to provide nutrients to other tissues, and restoration of normal GI function is critical since the loss of digestive and absorptive capacity in the malnourished state can result in serious consequences [6].

RESPONSE TO DIET

The GI response to diet is mediated by neural and hormonal stimuli. An important part of the neural stimulation of GI function occurs during the cephalic phase of digestion, when food is seen and smelled. This response essentially prepares the GI tract for the presence of food. Neural stimulation, primarily mediated by the vagus nerve, elicits acid and pepsin secretion in the stomach, gallbladder contraction and secretion of enzyme-rich fluid from the pancreas. Neural reflexes are also known to be involved in mediating motility of both the small and large intestines.

GI smooth muscle responses to stimulation of non-adrenergic non-cholinergic inhibitory nerves are thought to be mediated by polypeptides, ATP, or another unidentified neurotransmitter [7]. Nitric oxide (NO) an inorganic, gaseous molecule appears to act as an inhibitory neurotransmitter on GI smooth muscle. The “nitrgic” nerves appear to play major roles in the control of smooth muscle tone, motility, and fluid secretion in the GI tract. This knowledge is currently revising our understanding of GI tract neural response. NO-induced GI smooth muscle relaxation is mediated, not only by cyclic GMP directly or indirectly via hyperpolarization, but also by cyclic GMP-independent mechanisms. Understanding nitrgic innervation

and responses in the GI tract may lead to a new understanding of GI tract regulation, physiology, and pathophysiology and may also demonstrate how this sort of smooth muscle control affects GI motility and intestinal absorption overall.

In addition to the innervation of the gut, the GI tract contains various hormones and regulatory peptides that can be released by the presence of food and elicit responses from organs in the gut, which is one of the richest endocrine organs in the body. Insulin and glucagon are examples of hormones that are released from the GI tract whose peripheral effects on organs other than those of the GI tract have been well studied. The potential effects of other gut hormones on non-GI tissues have not been well characterized. The distribution of hormones and regulatory peptides along the gut is regional as shown in [Table 12.3](#).

The release of hormones is mediated by the presence of protein or amino acids, fat, and carbohydrate in the GI tract. Protein is a potent stimulant of gastrin, cholecystokinin (CCK), and gastric inhibitory peptide (GIP) release; fat is a stimulant of CCK, secretin, and GIP release as well as enteroglucagon, neurotensin, and peptide YY release. It is also known that carbohydrate presence is involved in the release of GIP as well as the endocrine hormones, insulin, and glucagon and that the magnitude of carbohydrate exposed surface area is also important [8–10].

The pancreas contains a range of hydrolytic enzymes that digest the macromolecules, which are ingested in the diet. These enzymes include proteases (e.g., trypsinogen, chymotrypsinogen, proelastase, procarboxypeptidases), [alpha]-amylase, lipase, colipase, phospholipase, and cholesterol esterase. The proteases, and possibly the lipid digestive enzymes, are secreted from the pancreas in an inactive form and are activated in the small intestine by the action of enterokinase or trypsin. The secretion of an enzyme-rich fluid from the pancreas can be stimulated by the hormones gastrin, released from the stomach, and CCK, which is released from the duodenum [11,12]. Thus, during the cephalic and gastric phases of digestion, pancreatic secretion is initiated, and while nutrients are in the small intestine, CCK has an important role in sustaining sufficient enzyme secretion for digestion to occur.

Dietary constituents can also stimulate enzyme secretion from the pancreas by feedback mechanisms. For example, dietary composition and content can stimulate pancreatic enzyme secretion via a vagal cholinergic pathway. This response is mediated by differences in the release of GI hormones [13,14].

The effect of diet on the secretion of digestive enzymes other than the proteases differs. The pancreas can adapt to a high fat, low carbohydrate diet by increasing the activity of lipase and decreasing the activity of amylase [15]. The mechanism for this adaptation is not known but may be mediated by the absorption of lipid and carbohydrate digestion products rather than release of a specific hormone.

It has long been known that dietary proteins and fats are potent stimulants of GI function, but until recently, the carbohydrate portion of the diet, whether the digestible carbohydrates (starch and sugars) or the non-digestible polysaccharides associated with dietary fiber, have been viewed as somewhat neutral. However, it is now known that glucose is a potent releaser of GIP, which augments insulin release [9].

Some sources of dietary fiber, such as pectins and gums, have been associated with slower digestion and absorption of carbohydrates and fats due to their ability to

Table 12.3 Regional Distribution of Gastrointestinal Hormones

Origin	Hormone(s)	Function
Stomach	Gastrin	Acid release/pH regulation
Duodenum and proximal jejunum (Upper Small Intestine)	Cholecystokinin (CCK-A)	Stimulates pancreatic enzyme delivery
		Stimulates bile delivery from gall bladder
	Secretin	Bicarbonate release/pH regulation
	Gastric Inhibitory Peptide (GIP)	Inhibits gastric motility and acid secretion
		Enhances insulin release
	Motilin	Controls upper GI pattern of contraction
	Enkephalins	Opiate-like actions
	Bombesin-like immunoreactivity	Stimulates CCK and gastrin release
	Neurotensin	Modulates motility
		Relaxes the lower esophageal sphincter
Distal Intestine (Ileum and colon)		Blocks stimulation of acid and pepsin secretion
	Glucagon-like-peptide 1	Enhances the release of insulin in response to glucose
	Glucagon-like-peptide 2	Stimulation of intestinal cell proliferation
	Oxyntomodulin	Inhibition of gastric secretion and motility
		Inhibition of pancreatic secretion
	Peptide YY	Regulates appetite control/Obesity regulation
Pancreas	Insulin	Regulates carbohydrate metabolism
	Glucagon	Blood glucose regulation
	Pancreatic Polypeptide	Increases gastric emptying and secretion
		Relaxes pyloric and ileoceccolic sphincters
		Relaxes the gall bladder and colon
	Somatostatin	Inhibits release of GIP, motilin, secretin, gastrin, CCK-A
		Lowers gastric emptying rate
		Inhibits release of insulin and glucagon
	Vasoactive intestinal peptide	Smooth muscle relaxation
		Stimulates pancreatic secretion
Entire GI tract	Substance P	Pain (nociception)
		Vomit reflex
		Induces vasodilation
		Stimulates salivary secretion

interfere with digestive enzyme activity, to increase the viscosity and volume of the intestinal contents, and by binding micellar components [16,17]. Dietary fiber and undegraded starch have been shown to be primary sources of fermentable substrate for the microflora of the large intestine [18]. The products of dietary fiber fermentation, such as the short-chain fatty acids, can be absorbed and utilized by the body.

Increases in plasma triglycerides after a meal originates from both intestinal and hepatic sources [19,20]. In contrast, plasma cholesterol concentrations do not change significantly during the alimentary period after a meal [19–21].

NORMAL INTESTINAL ABSORPTION

During the process of digestion, large, naturally occurring carbohydrates, proteins, and fats are hydrolyzed by catalytic enzymes to simple sugars, amino acids, and fatty acids, respectively. These smaller compounds are more efficiently absorbed in the small intestine and provide the necessary building blocks and energy content to be used by the cells of the body. Besides the absorption of dietary nutrients, the intestinal mucosa functions in the absorption of water, electrolytes, vitamins, and minerals. Electrolytes, water, and metabolic substrates are absorbed into the blood and distributed to cells throughout the body for their use. Figure 12.1 shows how different dietary components are processed and absorbed by the intestines.

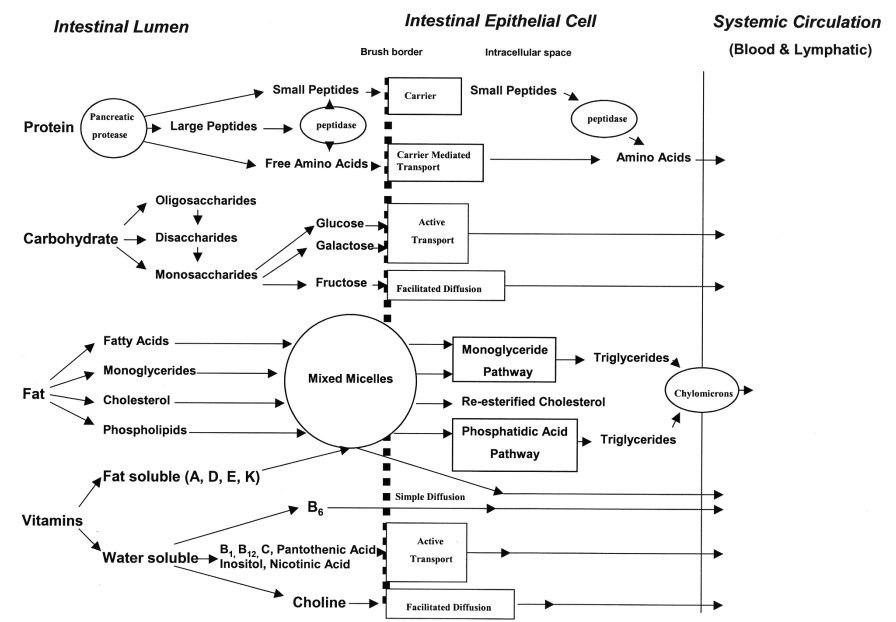


Figure 12.1 Intestinal processing and absorption of dietary components.

As shown in [Figure 12.1](#), intestinal absorption essentially involves the transport of nutrients from digested food and water from the gut lumen, across the intestinal epithelium, into the lymph or venous blood. Basic mechanisms of absorption into an intestinal cell are by three different mechanisms: simple diffusion, facilitated diffusion, and active transport. Diffusion does not use metabolic energy, and the solute is not transported against a gradient. It does not need a transport protein and velocity vs. substrate concentration is linear. Facilitated diffusion involves a transport protein, demonstrates substrate specificity, and the reaction is saturable. The solute is not transported against a gradient and does not need metabolic energy. Active transport involves transport proteins and demonstrates substrate specificity. Transport is against a concentration gradient and metabolic energy is necessary.

Most absorption takes place in the small intestine. The surface area of the intestine is well suited to its function: the structural adaptations of mucosal folds, villi, and microvilli give a total absorptive area for the small intestine of 200–500 m², or a 600-fold increase over the projected surface area of a smooth-surfaced tube of equivalent length. On average, for adults, total daily intestinal absorption amounts to between 200 and 300 g of carbohydrates, about 100 g of amino acids, about 100 g of fat, about 100 g of electrolytes, and 7 or 8 L of water. Under normal conditions and with few exceptions much of what is ingested is absorbed. It has been estimated that there is enough reserve capacity in the intestinal tract that five to ten times more food substances and two to three times more water could be absorbed per day in the small intestine over recommended average consumption [22].

The human small intestine villar absorptive surface and mucosal structure greatly increases the total surface area for highly efficient absorption. The lining of the stomach, in contrast, has a poor absorptive area because it lacks a villar absorptive surface and has tight junctions between its epithelial cells. Only alcohol and some drugs, such as aspirin, are absorbed here, and the amount of absorption is low.

The mucosa of the human small intestine has folds termed “folds of Kerckring” that increase the surface area of the mucosa about threefold. The millions of villi covering the surface of the mucosa enhance this absorptive surface area by perhaps another tenfold. Furthermore, each intestinal mucosal cell in the villus is characterized by a “brush border” that consists of approximately 600 microvilli per cell that substantially increases the total absorptive surface area of the small intestine perhaps another twenty-fold [23].

In addition to diffusion and active transport of digested materials across the cells of the intestinal epithelium, small amounts of substances are also taken into the cells via pinocytosis [22].

Actin filaments contract intermittently and cause continual movement in the microvilli, keeping mucosal cells exposed to new quantities of intestinal fluid enhancing the opportunity for absorption. Absorbed materials can be sent directly into the portal circulation or lymphatic system. Each intestinal villus contains a vascular system with an artery, a vein, blood capillaries, and a central lacteal for absorption into the lymph.

Fluids and Electrolytes

The small intestine is the primary site of fluid and solute absorption in the GI tract and absorbs approximately 85% of the fluid with the colon absorbing the remaining 15%. It is estimated that an adult human ingests about 2 L of fluid daily not including the volume of fluid from internal secretions. The secreted volumes are approximately three to four times the amount of fluid ingested or between 6 and 8 L of which approximate proportional amounts of the different components of secreted fluid are; saliva, 15%; gastric juice, 25%; pancreatic juice 10%; bile 20%; and intestinal secretions 30%. During GI absorption, the small intestine absorbs the majority of this fluid and the colon a lesser amount. It is estimated that of the total fluid entering the GI tract only 1% or less is excreted via fecal matter [22,24].

Water is transported through the intestinal membrane by osmosis. Partially digested solubilized food, “chyme” delivered from the stomach into the intestinal tract, is hypertonic and is adjusted to isotonicity in the duodenum. The opposite occurs when chyme is hyperosmotic. Water then is transferred to the chyme, making it isoosmotic with the blood plasma. When dissolved, substances are absorbed the osmotic pressure of the chyme is decreased. Water diffuses rapidly through the membranes of the intestine and is absorbed immediately into the blood with any associated ions. Water absorption by the jejunum is greater than the ileum; however, the colon is most efficient and absorbs more water than any other part of the GI tract.

Due to the handling of electrolytes the small intestinal mucosal cell may be considered a polarized epithelial cell. The plasma membrane consists of the brush border facing the lumen and the basolateral membrane facing blood. The $\text{Na}^+\text{-K}^+\text{-ATPase}$ is located exclusively on the basolateral membrane. Its function is to transport K^+ into the cell and Na^+ out of the cell. Both of these transport processes occur against a concentration gradient requiring metabolic energy. The $\text{Na}^+\text{K}^+\text{-ATPase}$ is a transport protein as well as an ATPase. The energy released after ATP hydrolysis is used to energize the transport. The stoichiometry between the transported ions is $3 \text{ Na}^+ : 2 \text{ K}^+$. This results in a Na^+ gradient and a K^+ gradient across the basolateral membrane.

It also produces a chemical gradient across the membrane (inside-negative). Under normal conditions, luminal fluid contains high concentrations of Na^+ (from diet, intestinal, and pancreatic secretions), so an electrochemical gradient is created across the brush-border membrane.

Carbohydrate Absorption

Luminal and membrane digestion of dietary carbohydrates produces glucose, galactose, and fructose. Fructose is absorbed by facilitated diffusion, so there is no need for metabolic energy, but a specific carrier is required. Once inside the cell, fructose is phosphorylated and converted to glucose prior to entering the portal blood.

Galactose and glucose are actively transported into the mucosal cell by a common transport protein [25]. The energy for this active transport is provided by the electrochemical Na^+ gradient. The glucose carrier has binding sites for Na^+ and

glucose. When both sites are occupied, it translocates across the membrane. The Na^+ and glucose are released into the cytoplasm of the cell and the unloaded, empty carrier comes back to the original position to start the transport cycle once again. Stoichiometry between Na^+ and glucose is 2:1 or 1:1. The transport of glucose and Na^+ via the carrier results in the transfer of a positive charge across the membrane. Both the electrical and chemical components of the electrochemical Na^+ -gradient provide energy for glucose transport. This process is known as Na^+ -glucose co-transport. Galactose shares the glucose binding site and can be absorbed in a manner similar to that outlined for glucose [25].

The electrochemical Na^+ gradient is the direct energy source for glucose transport, but ATP ultimately is necessary for the transport. The hydrolysis of ATP via the Na^+ - K^+ -ATPase system generates the electrochemical Na^+ gradient. In the intact cell, glucose transport is inhibited by metabolic poisons such as dinitrophenol (causes a reduction of ATP synthesis), hypoxia (synthesis of ATP is reduced), ouabain (Na^+ - K^+ -ATPase is inhibited) and phlorizin (blocks glucose from binding to its site on the carrier) [26,27].

Absorbed glucose leaves the cell across the basolateral membrane to enter mucosal capillaries. This process occurs via a facilitated glucose transport system, GLUT 2 [28]. The transporter is Na^+ independent and is inhibited by phlorizin. In a healthy human fed a meal containing 20–60 g of starch, as much as 10% of the starch metabolic product escapes absorption in the small intestine. This starch passes on to the colon, where it provides a carbon source for bacteria of the colon.

Amino Acids and Peptides

Intact proteins and large peptides are not appreciably absorbed in the human intestinal tract. Several different types of peptide and amino acid transport proteins have been characterized in the luminal membrane of intestinal epithelial cells [29–32]. Dipeptides and tripeptides are transported across the brush-border membrane. A single-membrane transport system with broad specificity is responsible for such small peptides. It has a high affinity for dipeptides and tripeptides with physiologic L-amino acids. This membrane transport system has greater affinity for peptides with amino acids containing bulky side chains [32].

The brush-border mucosal cells of the small intestine have a peptide transport system. This transport system is active and there may be multiple peptide transport systems. It is energized by an electrochemical H^+ gradient and not by a Na^+ gradient. It accepts dipeptides and tripeptides as substrates. The H^+ gradient across the brush-border membrane is generated by the action of the Na^+ - H^+ exchanger. The total amount of each amino acid that enters intestinal epithelial cells in the form of dipeptides and tripeptides is considerably greater than the amount that enters as a single amino acid.

Amino acids are transported across the brush-border plasma membrane into the intestinal mucosal cell by way of certain specific amino acid transport systems. There are three main sodium-dependent active transport systems for amino acids that occur in brush-border membranes. The neutral brush-border (NBB) system

transports most of the neutral amino acids, both hydrophobic and hydrophilic. The imino acid transport system handles proline and hydroxyproline. The PHE system transports phenylalanine and methionine. The dicarboxylic amino acid transporter transports aspartate and glutamate [33–35].

Two facilitated transport systems are independent of Na^+ . The Y^+ system transports basic amino acids, such as arginine and lysine, while the L system transports neutral amino acids, preferably those with hydrophobic side chains [36]. At the basolateral membrane are two different types of sodium-dependent transport systems. One system prefers small, hydrophobic amino acids and the other system prefers small neutral amino acids. The sodium-independent L system transports neutral hydrophobic amino acids [37]. This basolateral membrane is more permeable to amino acids than is the brush-border membrane. Therefore, diffusion is an important pathway for transport across the basolateral plasma membrane. This is especially true for amino acids with hydrophobic side chains.

Fat Absorption

Fat digestion products, i.e. monoglycerides, fatty acids, cholesterol, and phospholipids, are present in the intestinal lumen in the form of micelles (aggregates). Micelle formation is aided by the presence of bile acids. These products are absorbed into the intestinal mucosal cell from these micelles. On the surface of the brush border, pH is acidic due to secretion of H^+ from the cell by the Na^+H^+ exchanger. This acidic pH causes the dissociation of micelles and these products can now pass freely through the brush-border membrane by diffusion, largely dependent on lipid solubility. Free fatty acids, 2-monoglycerides, and other products of lipid digestion can diffuse across the brush-border membrane very rapidly. Cholesterol is absorbed more slowly than the other lipid products associated with micelles. As the micelles travel down the small intestine, they become more concentrated with cholesterol. Although the duodenum and jejunum are active in absorbing fatty components, most of the ingested fat is absorbed at the mid-jejunum.

The absorbed monoglycerides and free fatty acids are converted back into triglycerides within the intestinal cell. A recently discovered cytoplasmic fatty-acid-binding protein plays a role in transporting fatty acid to the smooth endoplasmic reticulum. This fatty-acid-binding protein has a higher affinity for unsaturated fatty acids than for saturated fatty acids, and it binds long-chain fatty acids more tightly than medium- or short-chain fatty acids. In the smooth endoplasmic reticulum, the 2-monoglycerides are re-esterified with fatty acids at the 1 and 11 carbons to reform triglycerides. Cholesterol is largely re-esterified, although some free cholesterol remains. Lysophospholipids are reconverted to phospholipids, such as lecithin. Some de novo synthesis of lipids can take place in intestinal epithelial cells [38]. Triglyceride synthesis occurs by two pathways: These are shown in [Figure 12.2](#) and are the

1. Monoglyceride Pathway
2. Phosphatidic Acid Pathway

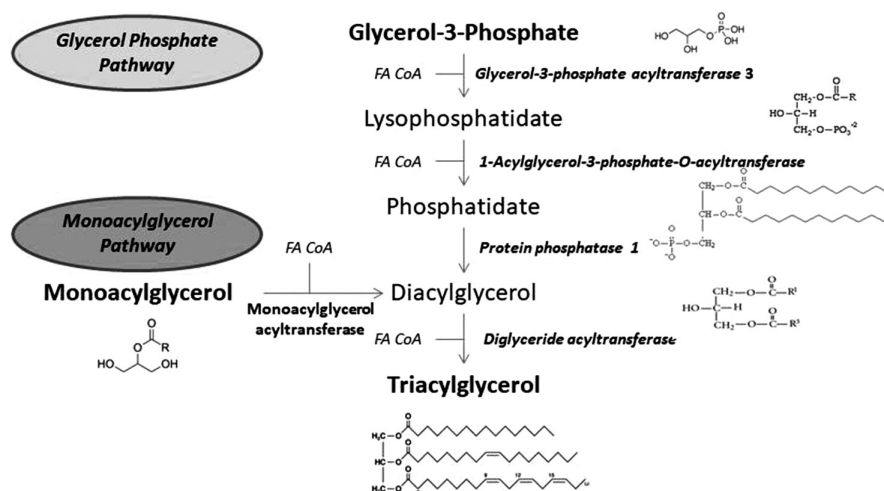


Figure 12.2 Triglyceride synthesis pathways.

The monoglyceride pathway accounts for about 70% of intestinal triglyceride synthesis. The reprocessed lipids and lipids that have been synthesized *de novo* accumulate in the vesicles of the smooth endoplasmic reticulum. Phospholipid covers the external surface of these droplets (vesicles). Their polar head groups face toward the aqueous exterior and hydrophobic acyl chains face the fatty interior. The lipid droplets thus formed, some as large as 1 mm in diameter, are known as chylomicrons. About 10% of the surface is covered by beta-lipoprotein, which is synthesized by intestinal epithelial cells.

The resynthesized triglycerides leave the cell in the chylomicron form. They form a milky fluid, the chyle, which diffuses into lymphatic lacteals, passes via lymphatic vessels to the thoracic duct, and into systemic circulation. Chylomicrons may now be as large as 750 nm in diameter. They are macromolecular particles containing 80% to 90% triglycerides, about 10% phospholipids, 3% cholesterol, and 2% protein. This protein needed for chylomicron synthesis is called apoprotein. If protein synthesis is inhibited in the intestinal cell, less chylomicron forms and triglycerides accumulate in the cytoplasm of the cell.

The majority of absorbed fatty acids larger than 10 carbon atoms are absorbed as esterified fatty acids in the lymph. Fatty acids less than 10 carbon atoms are transported into the portal venous blood. Glycerol and medium-chain fatty acids are water soluble and can be transported into blood without going through the chylomicron pathway [38].

Bile Acids

The ileum is the principal site of bile acid absorption. Bile acids cross the brush-border plasma membrane by simple diffusion or active transport. The active process is a secondary active transport powered by a Na^+ gradient across the brush-border

plasma membrane. Na^+ is cotransported with bile acids. Conjugated bile acids (bile salts) are substrates for active absorption. Deconjugated bile acids are less polar and can be absorbed by simple diffusion. Absorbed bile acids leave the intestinal cell via the basolateral membrane and enter the portal circulation. Hepatocytes take up the bile acids and reconjugate most deconjugated bile acids and rehydroxylate some secondary bile acids. These reprocessed bile acids, along with newly synthesized bile acids, are secreted into the bile [38].

Vitamins

Fat Soluble Vitamins

Fat-soluble vitamins (A, D, E, and K) partition into mixed micelles formed by bile acids and lipid digestion products. These vitamins diffuse across the brush-border plasma membrane of intestinal epithelial cells. Bile acids and lipid digestion products enhance the absorption of fat-soluble vitamins. Fat-soluble vitamins diffuse into intestinal mucosal cells and enter the chylomicrons that leave the intestine in the lymph. If bile acids are not present, a good proportion of fat-soluble vitamins leave the intestine in the portal blood. Vitamin A (retinol) is absorbed better than beta-carotene (provitamin A) and retinal. Beta-carotene and retinal are converted to retinol in the intestine. In the thoracic duct, vitamin A is present as fatty-acid esters of retinol. Vitamin A is absorbed independently of bile acid and leaves the intestine in the portal blood. Vitamin D is absorbed as the free vitamin in the jejunum. Many esters of vitamin D are hydrolyzed in the intestinal lumen before absorption. Fifty-five percent to 99% of ingested vitamin D is absorbed. The absorption of vitamin E requires the presence of bile-acid mixed micelles [38]. Although small quantities of ingested vitamin E are absorbed, if larger quantities are ingested a significant amount is not absorbed and will appear in feces. This vitamin also leaves the intestine in the lymph. Vitamin K1 and K2 contain hydrophobic side chains partitioned into bile-acid mixed micelles and leave the intestine through lacteals into lymphatic vessels. Vitamin K3 lacks a side chain and is absorbed independently of the mixed micelles. It leaves the intestine via portal blood.

Water-Soluble Vitamins

Most water-soluble vitamins, if consumed in high doses, are absorbed by simple diffusion. However, specific transport mechanisms also play an important role in absorption of these vitamins under other conditions. Vitamin C (ascorbic acid) is absorbed in the proximal ileum by active transport. Na^+ and ascorbate are co-transported into the cell [38]. The energy for this transport comes from the Na^+ electrochemical potential gradient. Biotin is transported by facilitated diffusion in the upper small intestine by a mechanism involving Na^+ presence in the lumen. Folic acid as 5-methyltetrahydrofolate is absorbed by simple diffusion [38,39]. Folic acid also is absorbed by carrier-mediated active transport via an H^+ gradient supplying energy in the jejunum. Thiamine (Vitamin B₁) is absorbed in free form by a

Na^+ -dependent active transport in the jejunum. Some thiamine is phosphorylated in mucosal cells of the jejunum. Riboflavin (Vitamin B_2) is absorbed in the proximal small intestine by facilitated transport; absorption is increased if bile acids are present. Pyridoxine (Vitamin B_6) is most likely absorbed by simple diffusion. Nicotinic acid also is absorbed by the jejunum by a Na^+ -dependent mechanism. Panthothenic acid and inositol are actively transported by the small intestine via a Na^+ gradient providing the energy. Choline absorption is by facilitated diffusion.

Vitamin B_{12} absorption involves an active transport process. Four physiologically important forms of vitamin B_{12} are cyanocobalamin, hydroxycobalamin, methylcobalamin, and deoxyadenosylcobalamin. Up to 5 mg of Vitamin B_{12} is stored in the liver. About 70% of the Vitamin B_{12} present in the bile is reabsorbed. This liver storage is thought to be sufficient for 3–6 years [37]. Most cobalamins are bound to proteins and absorbed in the intestine. The low pH of the stomach and pepsin release cobalamins; which are bound to R proteins, i.e., haptocorrin (HC) secreted from salivary glands and gastric juice. Intrinsic factor (IF) is a cobalamin-binding protein secreted by the gastric parietal cells [38]. Its secretory rate usually parallels that of HC. Dietary cobalamin bound to food proteins is released in the stomach by pepsin and acid pH and more free cobalamin binds to HC than to IF. The cobalamin-HC complex moves to the intestinal lumen, is digested by pancreatic proteases, and the liberated cobalamin now complexes with IF. The cobalamin-IF complex moves through the small intestine and binds to a transmembrane receptor (IFCR) in the ileum. After endocytosis of the complex, cobalamin is released intracellularly and transferred to transcobalamin II (TC II). This cobalamin-TC II complex leaves the ileal mucosal cell and enters the circulation.

Sodium and Chloride

The Na^+ - H^+ exchange system in the brush border of small intestinal and colonic cells is the principal means of Na^+ absorption from the intestinal lumen. This process is responsible for the acid pH microclimate at the surface of the brush-border membrane and transports Na^+ from the lumen into the cell and H^+ from the cell into the lumen. The electroneutral NaCl transport across the brush-border membrane of the small intestine is due to two exchangers: Na^+ - H^+ exchanger and the Cl^- - HCO_3^- exchanger. This is inhibited by acetazolamide, an inhibitor of carbonic anhydrase that reduces HCO_3^- within the cell. Na^+ is often co-transported with glucose, bile salts, amino acids, and water-soluble vitamins in the small intestine. In addition, a small amount of Na^+ may enter small intestine cells by passive diffusion [38].

The sodium pump, Na^+ - K^+ -ATPase, is responsible for active transport of Na^+ from the intestinal cell to the capillary. This pump is found in the basolateral membrane of the small intestinal cell. It is inhibited by ouabain, a cardiac glycoside.

Finally, in aldosterone-stimulated Na^+ transport, the transport occurs at the apical (luminal) membrane of the distal colon. This is due to a Na^+ channel that allows Na^+ to move passively down the electrochemical Na^+ gradient. Aldosterone stimulates the Na^+ channel. Chloride ions in the duodenum and jejunum are absorbed by passive diffusion.

Chloride and Bicarbonate

HCO_3^- secreted into the proximal duodenum moves to the jejunum, where both Cl^- and HCO_3^- are absorbed in large amounts. Most HCO_3^- is absorbed in the distal jejunum. Cl^- is absorbed and HCO_3^- is secreted in the ileum. Cl^- is absorbed and HCO_3^- is secreted in the colon [38].

Potassium

In the jejunum and in the ileum, the net flux of K^+ is from the lumen to the blood. As the intestinal contents are reduced through the absorption of water, the K^+ concentration increases and K^+ moves across the intestinal mucosa into the blood. Active transport of K^+ is absent in the small intestine, but in the colon K^+ may be secreted or absorbed. Usually K^+ is secreted in the colon [38].

Most K^+ absorption is due to its enhanced concentration in the lumen, caused by the absorption of water. K^+ loss may occur in diarrhea. If diarrhea is prolonged, the K^+ level falls in the body's extracellular fluid. Normal K^+ levels are needed for the heart to function properly, or cardiac dysrhythmias may occur.

Calcium

Abnormal or inadequate intestinal calcium absorption is a contributing factor in certain disease states, e.g., osteoporosis. The quantity of calcium absorbed in the intestine is controlled by how much calcium has been in the diet during recent periods of time. Calcium is absorbed by two distinct mechanisms, and their relative magnitude of importance is set by dietary calcium "history": Active, transcellular absorption occurs only in the duodenum when calcium intake has been low. This process involves import of calcium into the enterocyte, transport across the cell, and export into extracellular fluid and blood. Calcium enters the intestinal epithelial cells through voltage-insensitive channels and is pumped out of the cell via a calcium-ATPase.

The rate limiting step in transcellular calcium absorption is transport across the epithelial cell, which is greatly enhanced by the carrier protein calbindin, the synthesis of which is totally dependent on vitamin D [40–47].

Passive, paracellular absorption occurs in the jejunum and ileum, and to a much lesser extent, in the colon when dietary calcium levels have been moderate or high. In this case, ionized calcium diffuses through tight junctions into the basolateral spaces around enterocytes, and hence into blood. Such transport depends on having higher concentrations of free calcium in the intestinal lumen than in blood.

Calcium ions are actively absorbed throughout the small intestine, especially in the duodenum and jejunum, where Ca^{++} can be concentrated against a greater than tenfold concentration gradient. Although the absorption rate of Ca^{++} is greater than that of other divalent ions, it is 50 times slower than Na^+ absorption. Intestinal absorption of Ca^{++} is stimulated by hormonal vitamin D, 1,25-dihydroxycholecalciferol and parathyroid hormone. Ca^{++} moves from the brush border of the mucosal cell of the

small intestine, down its electrochemical potential gradient into the cytosol. This Ca^{++} is bound to an intestinal membrane calcium-binding protein (IMCa), which most likely is the transporter for Ca^{++} . The cytosol of the intestinal epithelial cells contains a cytosolic calcium-binding protein (CaBP) that binds two calcium ions with high affinity. CaBP is an essential component of Ca^{++} absorption. Its synthesis is stimulated by hormonal vitamin D (1, 25 dihydroxycholecalciferol). Free Ca^{++} and bound Ca^{++} are in dynamic exchange with Ca^{++} in the mitochondria and endoplasmic reticulum.

Within cells, Ca^{++} is stored in the mitochondria and endoplasmic reticulum, and it can be mobilized by the action of a secondary messenger inositol triphosphate. The basolateral cell membrane contains two transport proteins that are capable of ejecting Ca^{++} against its electrochemical potential gradient. Ca^{++} -ATPase in the basolateral membrane is the primary transport protein that splits ATP and uses the energy to transport Ca^{++} . The $\text{Na}^+/\text{Ca}^{++}$ exchanger uses this energy of the Na^+ gradient to exude Ca^{++} by active transport. Ca^{++} stimulates the activity of Ca^{++} -ATPase by binding to calmodulin, a calcium-binding protein. The calcium-calmodulin complex then stimulates a protein kinase that phosphorylates the Ca^{++} -ATPase, thereby activating it and enhancing its enzymatic and transport activities. Hormonal vitamin D is essential for normal calcium absorption and binds to nuclear receptors and stimulates messenger RNA that codes for a particular protein. Vitamin D induces the synthesis of Ca^{++} -binding protein, and the absorption of Ca^{++} into intestinal mucosal cells, and, at the level of the basolateral membrane, exocytosis out of the mucosal cell into the capillary. A $\text{Na}^+/\text{Ca}^{++}$ exchanger may also be involved at the basolateral membrane, but this is not regulated by hormonal vitamin D.

Iron

Iron homeostasis is regulated at the level of intestinal absorption, and it is important that adequate but not excessive quantities of iron be absorbed from the diet. Inadequate absorption can lead to iron-deficiency disorders such as anemia. On the other hand, excessive iron is toxic because mammals do not have a physiologic pathway for its elimination.

Iron is absorbed by villus enterocytes in the proximal duodenum. Efficient absorption requires an acidic environment, and antacids or other conditions that interfere with gastric acid secretion can interfere with iron absorption.

Ferric iron (Fe^{+++}) in the duodenal lumen is reduced to its ferrous form through the action of a brush-border ferrireductase. Iron is co-transported with a proton into the enterocyte via the divalent metal transporter DMT-1. This transporter is not specific for iron, and also transports many divalent metal ions [42].

Once inside the enterocyte, iron follows one of two major pathways. Which path is taken depends on a complex programming of the cell based on both dietary and systemic iron loads:

Iron abundance states: iron within the enterocyte is trapped by incorporation into ferritin and hence, not transported into blood. When the enterocyte dies and is shed, this iron is lost [43].

Iron limiting states: iron is exported out of the enterocyte via a transporter (ferroportin) located in the basolateral membrane. It then binds to the iron-carrier transferrin for transport throughout the body.

Iron in the form of heme, from ingestion of hemoglobin or myoglobin, is also readily absorbed. In this case, it appears that intact heme is taken up by the small intestinal enterocyte by endocytosis. Once inside the enterocyte, iron is liberated and essentially follows the same pathway for export as absorbed inorganic iron. Some heme may be transported intact into the circulation [29,38,43,52].

Dietary iron contains both inorganic iron and heme. The duodenum actively absorbs both forms. Iron is released from heme inside the mucosal cell and transferred into the body as inorganic iron. Fe^{2+} is absorbed faster than Fe^{3+} because ferric iron is insoluble above pH 3, whereas ferrous iron remains soluble at pH 8 [43–45]. Dietary constituents such as phosphates, carbonates, and oxalates reduce iron absorption because they form insoluble complexes with iron. These iron complexes are more soluble at low pH. Therefore, HCl secreted by the stomach enhances iron absorption; ascorbate also promotes iron absorption. The quantity of iron in the body is maintained by controlled absorption from the duodenum. Iron deficiency enhances erythropoiesis, and hypoxia increases intestinal iron absorption. Iron is stored in the mucosal cell as a ferritin complex. When iron absorption is increased, no ferritin complex is formed, and the iron is rapidly delivered into the plasma. When iron absorption is depressed, more iron is trapped in the form of a ferritin complex and retained in the mucosal cell. When Fe^{2+} leaves the mucosal cell, transferrin in the circulating blood is the carrier that delivers it to other tissues via transferrin receptors in the plasma membrane [38].

Other Ions

Copper

There appear to be two processes responsible for copper absorption—a rapid, low capacity system and a slower, high capacity system, which may be similar to the two processes seen with calcium absorption. Many of the molecular details of copper absorption remain to be elucidated. Inactivating mutations in the gene encoding an intracellular copper ATPase have been shown responsible for the failure of intestinal copper absorption in Menkes' disease.

A number of dietary factors have been shown to influence copper absorption. For example, excessive dietary intake of either zinc or molybdenum can induce secondary copper deficiency states. Copper is absorbed in the jejunum with about 50% efficiency. Some copper is secreted in the bile bound to certain bile acids and then lost in the feces. Magnesium is absorbed from the entire length of the small intestine, with about half of the dietary intake being absorbed. Phosphate is absorbed all along the small intestine. Some phosphate may be absorbed by active transport [38,43,46].

Phosphorus

Phosphorus is predominantly absorbed as inorganic phosphate in the upper small intestine. Phosphate is transported into the epithelial cells by co-transport with sodium, and expression of this (or these) transporter(s) is enhanced by vitamin D [38].

Zinc

Zinc homeostasis is largely regulated by its uptake and loss through the small intestine. Although a number of zinc transporters and binding proteins have been identified in villus epithelial cells, a detailed picture of the molecules involved in zinc absorption is not yet in hand.

Intestinal excretion of zinc occurs via shedding of epithelial cells and in pancreatic and biliary secretions [47,48].

There are a number of nutritional factors that modulate zinc absorption. Some animal proteins in the diet enhance zinc absorption. Phytates from dietary plant material (including cereal grains, rice, and corn) can chelate zinc and inhibit its absorption. Subsistence on phytate-rich diets is thought responsible for a considerable fraction of human zinc deficiencies [47,48].

ABNORMAL INTESTINAL ABSORPTION

There are a wide variety of conditions and agents that can adversely affect intestinal absorption. These range from a variety of disease conditions that cause malabsorption of many nutrients to conditions that produce abnormal absorption of only one or a few specific nutrients [49–54].

A comprehensive treatise on all known causes of abnormal intestinal absorption in humans is beyond the scope of this chapter and some conditions may have little relevance to toxicology and are beyond the scope of this chapter. However, many of the major causes of abnormal intestinal absorption, including malabsorption syndrome, are presented below including selected naturally occurring agents and agents of abuse that may be taken into the GI tract. The effects of synthetic drugs, biomolecules, and other xenobiotics on intestinal absorption and function are covered elsewhere in another chapter of this text.

Malabsorption Syndrome

“Malabsorption syndrome” is typically characterized by weight loss and steatorrhea may occur from a number of conditions (see [Table 12.4](#)) that may be classified by mechanism or cause. Most of these conditions produce malabsorption of many or most nutrients but there are some conditions that cause malabsorption of specific nutrients and may or may not produce malabsorption syndromes [49,53,54]. Some of these are listed in [Table 12.5](#). Unless the condition is quite severe, in many

Table 12.4 Conditions That Cause Malabsorption Syndrome

Disease of the small intestinal wall	a-β-lipoproteinemia
	Blind-loop syndrome
	Celiac disease
	Gluten enteropathy
	Infiltrative disease—amyloid, lymphoma
	Intestinal lymphangiectasia
	Jejunal resection
	Small bowel ischemia—atherosclerosis vasculitis
	Myotonic dystrophy
	Celiac sprue
	Tropical sprue
	Whipple's disease
Drugs	Alcohol
	Cathartics
	Cholestyramine
	Clofibrate
	Colchicine
	Neomycin
	Phenindione
	P-aminosalicylate
Insufficient bile acid	Extrahepatic biliary obstruction
	Intestinal stasis syndromes
	Intrahepatic biliary obstruction
	Short bowel syndrome
Insufficient pancreatic enzyme activity	Chronic pancreatitis
	Cystic fibrosis
	Enterokinase deficiency
	Isolated lipase deficiency
	Pancreatic carcinoma
	Pancreatic resection
Conditions requiring further diagnosis or mechanism unknown	Adrenal insufficiency
	Crohn's disease
	Bacterial enteritis
	Food anaphylaxis
	Carcinoid
	Diabetes mellitus
	Irritable bowel syndrome
	Hyperthyroidism
	Immune deficiencies
	Mast cell disease
	Parasitic infections (i.e., giardiasis, cryptosporidiosis)
Multiple defects	Gastrin-secreting tumor
	Ileal dysfunction
	Radiation enteritis
	Scleroderma
	Steatorrhea following gastric surgery

cases the large amount of reserve capacity within the GI tract for nutrient absorption often may compensate to a substantial degree and normal health continues. This can make the selection and interpretation of various tests for the diagnosis malabsorption sometimes difficult and challenging.

Table 12.5 Conditions That Cause Malabsorption of Nutrients

Amino acid malabsorption	Cystinuria Hartnup's Disease Methionine malabsorption Proline malabsorption
Folic acid malabsorption	Alcohol Dilantin Oral contraceptives
Monosaccharide malabsorption	Fructose malabsorption Glucose malabsorption
Primary disaccharidase deficiency	Acquired lactase deficiency Congenital lactase deficiency Congenital sucrase-isomaltase deficiency
Vitamin B ₁₂ malabsorption	Alcohol Congenital B ₁₂ malabsorption Pernicious anemia

In general, the digestion and absorption of food materials can be divided into three major phases.

1. The *luminal phase* is the phase in which dietary fats, proteins, and carbohydrates are hydrolyzed and solubilized by secreted digestive enzymes and bile.
2. The *mucosal phase* relies on the integrity of the brush-border membrane of intestinal epithelial cells to transport digested products from the lumen into the cells.
3. The *postabsorptive phase*, reassembled lipids and other key nutrients are transported via lymphatics and portal circulation from epithelial cells to other parts of the body.

Perturbation by disease processes in any of these phases frequently results in malabsorption.

Most patients with malabsorption have diarrhea and weight loss with perhaps other symptoms such as weakness, amenorrhea, bone pain, tetany, a tendency for bruising or bleeding, sore mouth, or tongue or peripheral neuritis. Since conditions that could produce these symptoms and signs are numerous and may or may not involve malabsorption, macroscopic, and microscopic stool examination is generally a part of any investigation of malabsorption.

The stool is examined for the presence of undigested material, particularly meat fibers and the presence of fecal fat by counting lipid droplets in the stool and by using quantitative analysis procedures from a 72-hr stool collection when the patient has been ingesting between 50 to 100 g of fat per day at least 48 hours before initiating the collection [55,56]. This test is commonly called the “stool fat test” and is based on the assumption that when secretions from the pancreas and liver are adequate, emulsified dietary fats are almost completely absorbed in the small intestine. When a malabsorption disorder or other cause disrupts this process, excretion of fat in the stool increases. The test evaluates digestion of

fats by determining excessive excretion of lipids in patients exhibiting signs of malabsorption, such as weight loss, abdominal distention, and scaly skin [55]. Reference values may vary from laboratory to laboratory but are generally found within the range of 5–7 g/24 hours for the normal human. Measuring the difference between ingested fat and fecal fat and expressing that difference as a percentage results in a “fat retention coefficient.” This fat retention coefficient is 95% or greater in healthy children and adults. A low value is indicative of steatorrhea [56].

Increased fecal fat levels are found in cystic fibrosis, malabsorption secondary to other conditions like Whipple’s disease or Crohn’s disease, maldigestion secondary to pancreatic or bile duct obstruction, and “short-gut” syndrome secondary to surgical resection, bypass, or congenital anomaly [57].

The management of patients with malabsorption addresses two principal factors: (1) the correction of nutritional deficiencies and (2) the treatment of causative disease, if possible. With regard to nutritional deficiencies the following are considered most important [57,58]:

- Supplementation with essential vitamins and minerals, such as calcium, magnesium, iron, and vitamins, which may be deficient
- Caloric and protein replacement
- Medium-chain triglycerides administration as fat substitutes as they do not require micelle formation for absorption and their transport is portal rather than lymphatic
- Parenteral nutrition in severe cases of intestinal disease

The treatment of causative disease may include the following as necessary.

- Consumption of a gluten-free diet to help correct celiac disease
- Consumption of a lactose-free diet to correct lactose intolerance
- Administration of protease and lipase supplements for pancreatic insufficiency
- Administration of antibiotics is the treatment for bacterial overgrowth
- Administration of corticosteroids and other anti-inflammatory agents to treat regional enteritis

Other Tests for Malabsorption

Since most available methods for measuring intestinal fat absorption have a common limitation in that the results depend on digestion as well as on absorption of the ingested lipid. These techniques have other practical disadvantages when applied to infants and children because they may require adjustment of the amount of fat consumed and because the test requires a few days to complete, loss of stool collection can be a problem. There is also considerable variability in the recommended dosage and in the interpretation of what constitutes an abnormal response.

Table 12.6 lists additional tests that may be useful in the investigation of malabsorption conditions. References are provided for further information with respect to how each test is conducted.

Table 12.6 Other Tests Used to Investigate Malabsorption Conditions in Humans

Test	Basis	References
D-xylose test	Proximal intestine primarily absorbs D-xylose; documents the integrity of the intestinal mucosa	[41]
Small bowel biopsy	Direct histopathological evaluation	[122]
Fructose breath test	Detects fructose malabsorption as unabsorbed fructose that results in elevated hydrogen content in expired air from microbial degradation in the colon	[123,124]
Schilling test	Evaluates Vit. B ₁₂ absorption using radiolabelled B ₁₂	[125,126]
Double-contrast small bowel enteroclysis radiographic study	Detects short gut, giant diverticula, fistulae, etc. detection by radiographic imaging	[130,131]
Video capsule endoscopy	Detects small bowel Crohn's disease using capsule imaging technology, capable of detecting limited mucosal lesions	[128,129,137]
Magnetic Resonance Imaging (MRI)	Detects small-bowel Crohn's disease using capsule imaging technology	[128]
CT scan of the abdomen	Detects evidence of chronic pancreatitis; enlarged lymph nodes are seen in Whipple's disease and lymphoma, intussusception	[131,132]
¹⁴ C-triolein breath test	Amount of label expired as ¹⁴ CO ₂	[127]
Endoscopic retrograde cholangiopancreatogram (ERCP)	Detects malabsorption due to pancreas or biliary-related disorders	[133]
Lactose/Hydrogen breath test	Detects lactose malabsorption as unabsorbed lactose results in elevated hydrogen content in the expired air	[134,135]
¹⁴ C-Bile salt breath test	Determines the integrity of bile salt metabolism	[136]
Upper GI endoscopy	Direct visual with biopsy for mucosal histopathological examination	[137–139]
Multidetector-row helical CT enteroclysis	Workup of patients with symptoms of intermittent small bowel obstruction, history of prior abdominal surgery for malignant tumor, history of radiation treatment	[131]

Carbohydrate Malabsorption Syndromes

Lactose malabsorption syndrome is due to a lack of lactase in the brush border of the duodenum and jejunum. These individuals are lactose intolerant. At least 50% of human adults are lactose intolerant. Such lactose intolerance is almost universal in oriental countries. To circumvent this condition, lactose-intolerant individuals simply avoid using dairy products. One also can take lactase pills along with dairy products and get along quite well. Some infants have rare congenital lactose intolerance. If they are fed a formula rich in sucrose or fructose, they do well [59].

Sucrose-isomaltase deficiency in the small intestine is an autosomal recessive inherited disorder. Significant portions of the Inuit population in Greenland have this

type of a sucrose-isomaltase deficiency. If placed on low-sucrose diets, these individuals show few problems. There is also a glucose-galactose malabsorption syndrome that is due to a defect in active transport for these monosaccharides, although fructose is well tolerated and can be substituted [60].

Protein Malnutrition

Protein-energy malnutrition (PEM) is associated with atrophy of the gastric mucosa and pancreas and morphological changes in the intestine. Intestinal changes are not distinguishable from morphological and functional abnormalities termed “sprue” or tropical enteropathy since its highest prevalence is in several tropical locations lacking adequate sanitation [61,62]. Clinically these changes may present as hypo- or achlorhydria, bacterial proliferation in the stomach and upper intestine and malabsorption. Acute and chronic diarrhea and tropical enteropathy, often with colonization by enteric pathogens and subclinical malabsorption, are often superimposed on PEM.

Malabsorption associated with acute, infectious diarrheas can result in the loss of 7% of yearly food energy; subclinical malabsorption nutrient losses in adults might equal 4%–6% of yearly food energy. The consequences vary with the adequacy of nutrient intake and presence or absence of concurrent infection. Protein-energy interactions include: (1) at any given energy intake, increasing protein will improve nitrogen retention until physiologic needs for nitrogen balance are approached; (2) at any given protein intake, increasing energy will improve nitrogen retention; (3) the efficiency of nitrogen retention will parallel the degree of malnutrition; and (4) in the hypermetabolic phases of infection, because of increased catabolism, there will be less nitrogen retention for any given energy and protein intake. Special attention should be given to situations where caloric intake is normal but protein intake is inadequate, because this has been associated with more marked intestinal morphological changes than when both protein and caloric intakes are decreased. Outcomes in investigations designed to assess nutritional needs in clinical or subclinical malabsorption should be evaluated under usual living conditions and dietary intake to avoid erroneous conclusions regarding prevalence or nutritional consequences.

Whipple's Disease

Whipple's disease is a rare infectious disease caused by bacteria, *Tropheryma whipplei*. It can affect any system of the body but occurs most often in the small intestine and interferes with the body's ability to absorb certain nutrients. Whipple's disease causes weight loss, incomplete breakdown of carbohydrates or fats, and malfunctions of the immune system. Diagnosis is based on symptoms and the results of a biopsy of tissue from the small intestine or other organs that are affected. When recognized and treated with antibiotics, Whipple's disease can usually be cured. Untreated, the disease may be fatal. Full recovery of the small intestine may take years and relapses are not uncommon [63].

Crohn's Disease

Crohn's disease is an inflammatory bowel disease (IBD) that causes chronic inflammation of the intestinal tract. It is estimated that 500,000 Americans have this condition. It is similar to another common IBD, ulcerative colitis [64,65].

Crohn's disease and ulcerative colitis are often mistaken for one another. Both cause many of the same symptoms which include Diarrhea, abdominal pain and cramping, bloody stool, ulcers, reduced appetite, and weight loss.

Crohn's disease begins with inflammation, most often in the lower part of the small intestine (ileum) or in the colon, but sometimes in the rectum, stomach, esophagus, or mouth. Unlike ulcerative colitis, in which inflammation occurs uniformly throughout an affected area, Crohn's disease can develop in several places simultaneously, with healthy tissue in between. In time, large ulcers that extend deep into the intestinal wall may develop in the inflamed areas.

A virus or bacterium may cause Crohn's disease and inflammation occurs when the immune system responds to the infection. The microorganism may also be more directly causal to the inflammatory response. *Mycobacterium avium* subspecies paratuberculosis (MAP), a bacterium that causes intestinal disease in cattle, has been suggested as a possible candidate because MAP is often isolated from the blood and intestinal tissue of patients with Crohn's disease, but only rarely in people with ulcerative colitis. It is also thought genetic susceptibility may trigger an abnormal response to the bacteria in some people, whereas it is also possible that the disease is caused by an abnormal immune response to bacteria present in the normal intestinal microflora. Approximately 20% of those with Crohn's disease have a parent, sibling, or child who also has the disease. Mutations in a gene, NOD2/CARD15, occur with significant frequency in people with Crohn's disease and appear to be associated with the early onset of symptoms as well as a high risk of relapse following surgery for the disease. Demographically, Crohn's disease occurs more often among people living in urban areas and in industrialized nations, therefore factors associated with such environments such as a diet high in fat or refined foods may also have some involvement [66].

Aging

As people age, gastric motility volume and acid content of gastric juice diminish. This causes hypochlorhydria (insufficient hydrochloric acid) and delayed gastric emptying. Intestinal absorption, motility, and blood flow decrease, impairing drug absorption. There is a proportionate decline in lean mass and blood volume. This results in higher serum drug levels in the elderly if not dosed appropriately [67–69].

Geriatric patients appear to be at increased risk for developing gastrointestinal problems associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs). It has been estimated that 3% to 4% of patients aged 60 or older using NSAIDs develop gastrointestinal bleeding, as compared with 1% of the general population [67,70,71].

Effects of Toxic Substances

Toxins

Various naturally-occurring toxins can affect intestinal absorption. These toxins can be of microbial origin or from fungal, plant, or animal sources.

Diarrheal diseases caused by microorganisms and their toxins are a major cause of mortality and morbidity throughout the world [72–77]. Acute diarrhea characterized by increased intestinal secretion is commonly a result of infection with enterotoxin producing organisms (enterotoxigenic *Escherichia coli*, *Vibrio cholera*, etc.) or due to decreased intestinal absorption from infection with organisms that damage the intestinal epithelium (enteropathogenic *E. coli* sp., *Shigella* sp., *Salmonella* sp.) Most of the bacterial toxins exert their effect through involvement of ADP-ribosylation proteins; otherwise essential for several cellular functions while other toxins involve guanylate cyclase systems or calcium and protein kinases for their ultimate action. Many of these toxins are of microbial origin and play significant roles in enteral infectious disease outbreaks. For example, cholera toxin affects the human jejunum by reducing the absorption of water and electrolytes progressively and induces secretion in a dose dependent fashion [72,76].

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) colonizes the large intestine causing a spectrum of disorders, including watery diarrhea, bloody diarrhea (hemorrhagic colitis), and hemolytic-uremic syndrome [73]. Stx is a multimeric toxin composed of one A subunit and five B subunits. Stx2 B subunit induces fluid accumulation independently of A subunit activity by altering the usual balance of intestinal absorption and secretion toward net secretion.

Clostridium perfringens type A produces a 35 kDa enterotoxin (CPE) that is an important cause of food poisoning, human non-foodborne GI disease, and some veterinary GI diseases. CPE action involves formation of complexes in mammalian plasma membranes. One such complex of approximately 155 kDa is responsible for plasma membrane permeability alterations, which result in enterotoxin-treated mammalian cell death. Such membrane permeability changes also damage the epithelium, allowing the enterotoxin to interact with the tight junction (TJ) protein occludin. CPE:occludin interacts to form an approximately 200 kDa CPE complex and the internalization of occludin into the cytoplasm. Removal of occludin (and possibly other proteins) damages TJs and disrupts the normal paracellular permeability barrier of the intestinal epithelium, which may contribute to CPE-induced diarrhea. Low CPE doses kill mammalian cells by inducing a classic apoptotic pathway involving mitochondrial membrane depolarization, cytochrome C release, and caspase 3/7 activation. High enterotoxin doses, however, induce oncosis, which is a proinflammatory event. CPE is a unique, multifunctional toxin with cytotoxic, TJ-damaging, and potentially significant proinflammatory action [74].

Botulism occurs from consumption or inhalation of preformed botulinum toxin or growth of *Clostridium botulinum* bacteria in the GI tract, or within a wound. Growth of *C. botulinum* in the GI tract releases botulinum toxin that reaches the

circulation. All forms of botulism cause progressive weakness, bulbar signs (blurred vision, diplopia, mydriasis, dysphagia, and dysarthria), and respiratory failure with normal sensation and mentation. Patients can recover normal muscle strength within weeks to months, but usually complain of fatigue for years [75].

Fungal toxins, such as amatoxins and orellanine, can cause severe organ damage in the human body. Amatoxins are bicyclic octapeptides, occurring in some *Amanita*, *Galerina*, and *Lepiota* species, that induce deficient protein synthesis resulting in cell death and also may exert toxicity through inducing apoptosis. Target organs are intestinal mucosa, liver, and kidneys. Poisoning generally results in dehydration and electrolyte imbalance, liver necrosis, and possibly kidney damage. Amatoxins from *Amanita phalloides* and related species of mushrooms are associated with severe morbidity and a high mortality rate. Circulating amatoxins can be detected in the serum of poisoned patients as long as 30 hours after ingestion. Toxic effects are particularly high in susceptible cells, such as hepatocytes. The administration of cathartics, adsorbent agents, and gastroduodenal lavage are of value in preventing further absorption of toxins from the GI tract [77,78].

Recently, the number of blooms of algae that produce toxins has increased in frequency, intensity and geographical distribution, and illnesses resulting from toxins from marine algae, fish, and shellfish contaminated with toxins may also be increasing. Scombrototoxic poisoning from toxins in the tissues of certain fish is currently the most common cause of food poisoning associated with the consumption of fish and shellfish. Poisoning results from the consumption of spoiled fish of the families Scomberesocidae or Scombridae—in particular tuna, mackerel, skipjack, and bonito—which naturally contain high levels of histidine. Incorrect storage of fish allows bacterial histidine decarboxylase to convert histidine to histamine. The ensuing symptoms are thought to result from the ingestion and GI absorption of large amounts of histamine [79,80].

Pharmaceuticals and Toxicants

There are many pharmaceutical agents and toxic chemicals that can affect intestinal absorption and to attempt to list or discuss them all is beyond the scope of this chapter and will be discussed in another [chapter 10](#) of this text. Some are encountered unintentionally or by accident and the result is intoxication while others are employed pharmacologically to affect some form of localized intestinal effect or absorbed by any of the different avenues of absorption discussed previously to systemically distribute and act therapeutically.

Some of examples of agents that affect intestinal absorption significantly are provided as follows:

- Acetazolamide—inhibitor of carbonic anhydrase in intestinal epithelial cells involved with HCO_3^- production
- Belladonna (atropine)—competitive antagonist of acetylcholine at muscarinic receptors, antisecretory agent, decreases GI motility
- Clonidine—alpha adrenergic stimulating agent with antisecretory and antidiarrheal

- Domperidone—blocks inhibitory effects of dopamine and increases GI motility
- Ezetimibe—cholesterol absorption inhibitor that blocks the translocation of dietary and biliary cholesterol from the gastrointestinal lumen into the intracellular space of jejunal enterocytes [80,81]
- Histamine₂ receptor antagonists (cimetidine, ranitidine, famotidine)—reduce gastric acid output that may affect subsequent intestinal absorption processes
- Loperamide—opioid receptor agonist, antisecretory agent that slows colonic motility
- Opiates—antisecretory effects from relaxation of intestinal smooth muscle
- Ouabain—inhibits the Na⁺-K⁺-ATPase pump of intestinal epithelial cells
- Prokinetic drugs (cisapride, metoclopramide, erythromycin, bethanechol)—increase GI motility in selected portions of the GI tract or less selectively over the entire tract depending on pharmacologic action and mechanism of the agent
- Proton pump inhibitors (lansoprazole, rabeprazole, esomeprazole, omeprazole)—inhibits gastric acid secretion that may effect subsequent intestinal absorption and motility events
- Somatostatin—regulatory peptide that inhibits the release of insulin and glucagon from the pancreas and exerts a general inhibitory effect on many other GI hormones including gastrin, gastric inhibitory peptide, cholecystokinin, secretin, vasoactive intestinal polypeptide, and motilin; hormonal action reduces exocrine secretions and slows digestion, decreases GI motility, and decreases absorption of nutrients

Effects of Naturally Occurring Stimulants, Depressants, and Substances of Abuse

Alcohol

There are many alcohols that could reach their way into the intestinal tract either intentionally or unintentionally. Common alcohols include ethanol, isopropanol, and methanol.

Ethanol, ethyl alcohol, can be used as a solvent, an antiseptic, or a beverage. Alcoholic beverages such as beer, wine, and distilled spirits all contain ethanol. It is the single most widely used drug in the world and therefore has been studied extensively in its effect on the digestive system. It may cause decreased absorption of D-xylose, folic acid, and thiamin. In alcoholics it may cause decreased absorption of essential nutrients such as B₁₂ and methionine.

The toxicity of isopropanol is almost twice that of ethanol in which symptoms of poisoning may include catatonia and ketonuria with the loss of metabolic acidosis.

Methanol is generally considered non-toxic and can be found in items such as anti-freeze, fuel, solvent, and paint remover.

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Ethyl alcohol is one of the most commonly abused drugs with consumption averaging 10 L per person per year in the United States. Alcohol is distributed throughout the tissues of the body by means of water; therefore, major organs of the body such as the heart and the brain receive the same blood alcohol concentration. Alcohol is not digested; instead it is absorbed into the mucus linings of the digestive system. Rate of absorption differs depending on different factors such as body size, sex, and concentration of alcohol. Alcohol dehydrogenase, the principal enzyme involved in the metabolism and detoxification of alcohol is typically less abundant in women and therefore some women may have less resistance to the effects of alcohol versus some men. Carbonated drinks contain carbon dioxide that appears to affect the gastrointestinal epithelium in a manner that results in a higher rate of alcohol absorption. Mucosal absorption begins within 10 minutes after the first sip of the beverage and absorption is completed in the small intestine. The majority of absorption (80%) occurs in the small intestine because of its large surface area and concentration of villi and microvilli. More specifically, the duodenum and jejunum contain the largest surface area and the ileum contains the least amount of surface area. If large quantities of alcohol are consumed, it may lead to impaired intestinal absorption of nutrients, vitamins and sodium, and water that may lead to diarrhea. Additionally, alcoholics may be inhibited from absorbing other nutrients such as B₁₂, folic acid, thiamin, amino acids, calcium, and magnesium. Chronic alcohol consumption is associated with increased risk of major gastrointestinal cancers including cancer of the esophagus, stomach, and colon [82]. The consumption of alcohol may also inhibit the absorption of some drugs such as cephalosporin, chlorpropamide, sulfonamides. In addition, metabolism of antihistamines, barbiturates, and narcotics may be slowed with alcohol intoxication. Damage to the mucus of the digestive system due to alcohol consumption may lead to duodenal erosions and bleeding. It can also lead to greater permeability that may allow bacterial toxins to enter the blood from the digestive tract that may cause additional damage to digestive as well as other organs. Another consequence of excessive consumption of alcohol is the relaxation of the esophageal sphincter which deters gastric contents from damaging the esophageal mucus. Alcohol reduces the pressure of the esophageal sphincter which results in increased heartburn. Alcoholics in particular have more relaxed esophageal muscles and thus have a greater increase in acid reflux and are more prone to esophageal tears due to vomiting. Membranes in the mouth may become irritated due to consumption of alcohol and eventually cause throat or mouth cancer. Consumption of alcohol has also been linked to an increased risk of tumors in the pharynx, colon, mouth, stomach, and esophagus. Tumors may also develop in the gastrointestinal track due to free radicals such as preservatives and additives that can be found in alcoholic beverages. The risk of developing these cancers may increase linearly with the addition of tobacco usage.

Low-alcohol-containing beverages such as wine and beer, when consumed in small amounts, may contain substances that may induce gastric motility. Higher quantities may delay gastric emptying and thus cause a feeling of fullness or nausea [83]. Appetite is increased with consumption of small quantities of alcohol because it stimulates the production of stomach juices, over time, appetite may become dulled which could lead to malnutrition. An influx in gastric juices due to alcohol consumption may also lead to the onset of ulcers on the stomach lining.

Betel

In counties in south and Southeast Asia it is common to chew a combination of betel, areca nut, and tobacco packaged into “quids.” Nuts of the Areca palm are wrapped in leaves and spread with lime paste to form small packets that can be inserted into the mouth. In some populations, tobacco may be inserted. Betel chewing is the fourth most common habit in south Asia behind smoking, alcohol, and caffeine usage. Some diseases are linked to the use of betel chewing such as oral submucous fibrosis, oral leukoplakias, oral cavity, and head and neck cancers [84]. Betel chewing may lead to the development of esophageal cancer and may raise the carcinogenetic effect of smoking and alcohol.

Although the effects of betel on the development of cancer is well known, the effects of betel quids on vitamin D metabolism and calcium homeostasis in the gastrointestinal tract are only just now being studied, and it is likely that this practice has other effects on intestinal motility and absorption that are worthy of study given the commonality of use in some parts of the world.

Caffeine

Caffeinated coffee stimulates colonic motor activity with its magnitude being similar to that of a meal. It is 60% stronger than water and 23% stronger than decaffeinated coffee [85].

Coffee contains a multitude of substances, many of which are potentially biologically active although the main physiologic effects resulting from its consumption are usually ascribed to the presence of caffeine. Coffee is also an extremely rich source of chlorogenic acids (CGAs), an important group of biologically active dietary phenols, the best known of which is 5-caffeoylquinic acid (5-CQA).

The daily intake of CGA by coffee drinkers ranges from 0.5 to 1.0 g (3, 6). Olthof et al. [84] showed that 33% of a 2.8-mmol load of CGA was absorbed by ileostomy patients. Plasma glucose concentrations were significantly higher after consumption of caffeinated coffee than after consumption of the control beverage or decaffeinated coffee.

Caffeine is an adenosine receptor antagonist [86–88] and inhibits muscular glucose uptake, even in the presence of insulin [88]. Moreover, Sharp and Debnam [86] have shown that acute luminal exposure of GI cells to cAMP has stimulatory effects on sugar transport.

The secretion of the hormones GIP and GLP-1 are significantly altered in response to the consumption of caffeinated beverages.

Cannabinoids

The human nervous system contains cannabinoid CB1 receptors that decrease the functions of the gastrointestinal track. CB1 receptors depress gastrointestinal motility, by inhibiting ongoing contractile transmitter release. This results in a relaxation of the sphincters of the lower esophagus that in turn retards gastric emptying

and inhibition of the transit of materials through the small intestine. The inhibitory effects of cannabinoid receptor agonists on gastric emptying and intestinal transit are mediated to some extent by CB1 receptors in the brain as well as by enteric CB1 receptors.

Acid production the stomach is also inhibited by the activation of CB1 receptors. In clinical trials, marijuana has been found to aid in colon functioning, intestinal dysfunction, and diarrhea. In the future, cannabinoids may be used to help treat gastrointestinal dysfunction, diarrhea, vomiting, nausea, colon cancer, and inflammation of the bowels [89].

There also evidence that cannabinoid receptor agonists can suppress increases in gastrointestinal activity precipitated by naloxone in morphine-dependent animals. Δ^9 -THC but not cannabidiol produces a dose-related blockade of naloxone-induced signs of heightened gastrointestinal activity (diarrhea and increased defecation) as well as other abstinence signs in morphine-dependent rats. These findings indicate that cannabinoids may have potential for the management of opioid withdrawal in human clinical settings [90].

Catechins

Recently, the benefits of drinking green and black tea on metabolism rates have been widely publicized [91–99]. Green and black tea both originate from the leaves of the *Camellia sinensis*. The leaves of tea plants contain large amounts of monomeric flavonoids called catechins. Green tea is made by inactivating the enzymes in the freshly picked leaves while black tea is produced from fresh green teas through a fermentation process. Tea catechins have been shown to reduce plasma cholesterol and suppress hypertriacylglycerolemia by reducing triglyceride absorption. However, the mechanism is not yet clear. One of the possible mechanisms is that tea polyphenols may modify dietary fat emulsification in the gastrointestinal tract. The digestive enzyme (lipase) acts on specific emulsion interface properties (droplet size and surface area). Therefore, changes in these properties may modify emulsification and lead to changes in dietary fat digestion and absorption. The effect of both green and black tea on the changes of emulsification has been examined *in vitro* by measuring the droplet size and the surface area [96,97]. Using a model emulsion system containing olive oil, phosphatidylcholine (PC), and bile salt developed to simulate small intestinal conditions initial changes in droplet size (from 1.4 to 52.8 μm and from 1.4 to 25.9 μm) of the emulsion were observed in the presence of 1.04 and 0.10 mg/mL of total catechins prepared from green and black tea, respectively. Both teas caused similar changes on the emulsion properties; however, black tea was more effective than green tea [93,95].

Flavonoids that are not absorbed in the small intestine are metabolized by the bacterial flora in the colon [93,99]. Colonic microorganisms mediate fission of the central C3 ring of catechins. This type of fission is decisive for the basic structure of the resulting metabolites, i.e., hydroxyphenyl-valerolactones and phenolic acids [97–99]. These metabolites are absorbed from the colon, and their urinary concentrations exceed that of the intact flavonoid [84,100–102]. In addition, dietary

flavonoids may have significant effects on the colonic flora [103] and thus confer a type of prebiotic effect. In this respect, it has been noted that not only tea drinking but also wine [100], cider [101], and coffee [85,88] consumption can result in increases in urinary hippuric acid excretion, indicating that polyphenols from different dietary sources may have similar effects on the colonic flora. Green tea consumption and black tea consumption result in similar amounts of microbial degradation products that are absorbed by the body. These microbial metabolites, and not the native tea flavonoids, may be responsible for at least some of the health effects attributed to tea consumption [99,100].

Cocaine

Historically, cocaine was utilized by Native Americans and other indigenous people because of its ability to suppress hunger and was taken orally by chewing on the leaves of the plant [102]. Current data suggests that there has been a rise in the number of deaths attributed to cocaine by means of oral ingestion [103]. A popular means of drug smuggling involves the swallowing of several balloons, condoms, or small vials that contain cocaine. This practice is commonly termed “body packing” and this procedure is also used to smuggle other drugs of addiction such as cannabis and heroin, however, most research on the effects of body packing has been conducted on individuals who smuggle cocaine. In the past it was assumed that cocaine is harmless when ingested orally [102]; however, current studies have shown quite the opposite [103,104]. On occasion, a body packer will discover that one of the swallowed vessels has ruptured in their stomach which may cause extreme complications in digestion and normal gastric functioning. Thirty minutes after ingestion cocaine begins to be absorbed into the gastrointestinal track and becomes ionized due to the acid contained in the stomach. The cocaine may pass through the stomach but it does not become appreciably absorbed until it reaches the more alkaline small intestine. After a ruptured package is discovered through x-ray or sonogram an emergency surgical exploration must occur, or in the absence of complications the individual may be administered laxatives. Complications due to ingestion of cocaine may include status epilepticus, wide and narrow complex bradyarrhythmias, ventricular arrhythmias and delayed hypothermia [103,104].

Although the most common application of cocaine is through the nasal passage, a study conducted by van Dyke and his colleagues [104] concluded that oral administration produced the quickest high (15–60 minutes) versus the intranasal application (45–90 minutes). The “high” experienced by users who chose intranasal application may be attributed to the passage of cocaine through the nasopharynx into the gastrointestinal tract.

Nicotine

Smoking has been found to have not only negative but also positive consequences on its effect on the gastrointestinal system. Cigarette smoking has a high risk factor for developing gastroduodenal ulcers and gastric carcinoma. Smoking may also tighten the gastric mucosa in smokers and is associated with a smaller increase of

gastric permeability induced by alcohol [105]. There are several causative agents which cause gastric damage which include cadmium and arsenic in particular [106]. Tobacco smoke has been shown to inhibit the production of growth factors, including epithelial growth factor (EGF) and vascular endothelial growth factor (VEGF), both of which inhibit cell proliferation and blood vessel generation [106]. Nicotine itself has been shown to increase the frequency of gastric tumors and double the frequency of tumor formation [107]. Nicotine has also been identified as an important risk factor for Crohn's disease and it has been proven that current smokers have double the lifetime risk of developing Crohn's disease compared to non-smokers [108]. While Crohn's disease is commonly seen in smokers, ulcerative colitis is rarely seen in smokers and therefore, smoking is considered a risk factor for ulcerative colitis, and [109] noted that 8% of patients with ulcerative colitis are smokers, compared to 24% of the general population [109]. The cause of this protective factor is still unexplained; however, several theories exist such as impairment in physical defenses, and changes to the inflammatory mediators in the intestinal mucosa [110]. Smoking is also an important risk factor for appendicitis, particularly in women [111].

Smokeless tobacco users consume a significantly larger amount of nicotine into the gastrointestinal tract because they may swallow small amounts of tobacco juice. The majority of the absorbed nicotine is converted to cotinine during first-pass hepatic metabolism [112]. Smoking can have a detrimental and beneficial effect on gastrointestinal disease—it has such a “polarizing effect” in patients with Crohn's disease and ulcerative colitis. Studies in tobacco smokers have made it difficult to identify which agents are responsible for these effects but work on the action of nicotine alone might help to explain some of the positive and negative links between smoking and gastrointestinal disease [113]. Unlike cigarette smokers, spit tobacco (ST) users absorb a significant amount of nicotine through the gastrointestinal tract while swallowing tobacco juice. The majority of the absorbed nicotine is rapidly converted to cotinine during first-pass hepatic metabolism. This process potentially compromises the utility of cotinine as a biomarker for systemic nicotine exposure in ST users. To investigate this question, we correlated nicotine and cotinine concentrations with clinical measures of ST use in 68 daily ST users enrolled in a nonnicotine pharmacologic intervention trial. We found that a higher frequency of swallowing tobacco juice ($P = 0.007$) was an independent predictor of higher serum cotinine concentrations. Serum nicotine concentrations, on the other hand, were not correlated with a higher frequency of swallowing. In the absence of a reliable way to measure frequency of swallowing, we conclude that cotinine should not be used for guiding clinical decisions that depend upon a precise quantification of systemic nicotine exposure, such as tailored nicotine replacement therapy [112].

Glycyrrhizic acid is widely applied as a sweetener in food products and chewing tobacco. In addition, it is of clinical interest for possible treatment of chronic hepatitis C. In some highly exposed subjects, side effects such as hypertension and symptoms associated with electrolyte disturbances have been reported. Glycyrrhizic acid is mainly absorbed after presystemic hydrolysis as glycyrrhetic acid. Because glycyrrhetic acid is a 200–1000 times more potent inhibitor of 11- β -hydroxysteroid dehydrogenase compared to glycyrrhizic acid, the kinetics of glycyrrhetic acid are relevant in a

toxicological perspective. Once absorbed, glycyrrhetic acid is transported and taken into the liver by capacity-limited carriers, where it is metabolized into glucuronide and sulfate conjugates. These conjugates are transported efficiently into the bile. After outflow of the bile into the duodenum, the conjugates are hydrolyzed to glycyrrhetic acid by commensal bacteria; glycyrrhetic acid is subsequently reabsorbed, causing a pronounced delay in the terminal plasma clearance. Pharmacokinetic modeling shows that, in humans, the transit rate of gastrointestinal contents through the small and large intestines predominantly determines to what extent glycyrrhetic acid conjugates will be reabsorbed. Parameters that can be estimated noninvasively may serve as a useful risk estimator for glycyrrhizic-acid-induced adverse effects because in subjects with prolonged gastrointestinal transit times glycyrrhetic acid might accumulate after repeated intake [114].

Clinical evaluation, upper gastrointestinal endoscopy and electron microscopy of mucosal biopsies from the antrum, body and fundus of stomach from control subjects and habitual tobacco chewers show marked differences. Electron microscopic abnormalities such as discontinuous, fragmented basement membrane with reduction in hemidesmosomes, and widened intercellular spaces filled with clusters of desmosomes were found in the gastric mucosa of habitual tobacco chewers; these were similar to those reported in experimental carcinogenesis and leukoplakia. It is concluded that habitual chewing of tobacco produces electron microscopic alterations in the human gastric mucosa which may be important precursors for gastric malignancy [115].

Electronic cigarettes (e-cigs) or “vaping” was introduced in the United States in 2007 and were advertised as a smoking cessation aid or alternative to tobacco use. Much attention has been paid to the short- and long-term effects of e-cigarettes. Concerns include the potential danger of highly concentrated nicotine and accidental ingestion. At the present time, there is no uniform guideline for production and marketing of these devices. It is difficult to identify the long-term health effects as few people have used these products for a prolonged time period [116]. Several studies have identified toxic chemicals in e-cigarette vapor including formaldehyde, acetaldehyde, nitrosamines, and heavy metals [117]. Additional research is needed to identify the long-term effects of e-cigarettes on gastrointestinal absorption.

Several smoking cessation aids exist such as nicotine patches, gum, and lozenges. Nicotine lozenges have been available over the counter for over 15 years. Studies on these lozenges have found that they contain a substantial amount of nicotine and may provoke irritation in the gastrointestinal tract that may result in vomiting [118].

Opium and Opioids

Opioid receptors in the gastrointestinal (GI) tract mediate the effects of endogenous opioid peptides and exogenously administered opioid analgesics, on a variety of physiological functions associated with motility, secretion, and visceral pain. Opium use has been shown to be associated with statistically significant increased risk of GI mortality, malignant causes of GI mortality, and non-malignant causes of GI mortality [119]. In particular, this research has shown an increased association between opium use and esophageal cancer mortality.

INTESTINAL ABSORPTION MODELING

Human intestinal absorption is an important roadblock in the formulation of new drug substances. In many cases obtaining direct information with respect to evaluating the intestinal absorption of new candidate drug compounds is either (1) time-consuming, due to the need for clinical study design and conduct; (2) expensive due to the cost of conducting clinical studies with several candidate compounds; or (3) potentially dangerous if the compound has not been well characterized toxicologically. For these reasons computational models are being increasingly developed and utilized for the rapid estimation and prediction of the human intestinal absorption of various substances and compounds. Generally, the initial parameter estimates are determined via *in vivo* animal experiments or *in vitro* permeability studies. Currently, permeability through human Caco-2 cells is a method in common use. There are several different methods in use for the development of predictive models and statistical quality is of importance in their selection and use [120]. As pharmaceutical and biotech companies strive to reduce the enormous costs and time required to bring new drugs to market, computational modeling methods have become an important and necessary part of drug discovery and development. These computational models range from simple spreadsheet routines to sophisticated supercomputer molecular dynamics models.

In general, modeling procedures to predict human intestinal absorption have one or more of the following objectives:

- Rapid analysis and understanding of the behavior of drug candidates in animals and human
- Rapid ability to test hypotheses regarding formulation, changing physicochemical parameters, fasted and fed state effects, ionization effects on solubility and absorption, and more
- Ability to quickly estimate best dosing for toxicity studies in animals
- Ability to fit absorption/pharmacokinetic models to Phase I data in human, and with those models, to determine optimum dosing for later phases

The ability of these models to predict human intestinal absorption depends on the quality of the input information but, in general, the ability to predict absorption with between 70% and 90% success on a qualitative basis using datasets from compounds that have known or subsequently determined human intestinal absorption data.

There are over 100 computational packages commercially available for the development of absorption models. However, some are used more widely than others and some claim to be “validated” which in quality-control terms typically indicates that each keystroke has been checked and each module has been tested with a standard data set and has returned a computed value within acceptable numerical limits. Currently, one popular software package, GastroPlus™ (Simulations Plus, Inc., Lancaster, CA), is widely used as are packages designed to make building physiologically based pharmacokinetic (PBPK) models for humans relatively simple by building in collections of human parameter data from the literature so that each model is built on a relatively similar set of initial physiological parameter estimates.

Commercial software packages for PBPK model development include commercial “turnkey” packages such as PBPK Modeling® (The Lifeline Group, Annandale, VA) and acslExtreme® (Aegis Technologies Inc., Huntsville, AL). Other computational methods employ various molecular structure-based approaches such as the topological sub-structural approach (TOPS-MODE) approach used by Pérez et al. [121]. In any case, these in silico methods of estimating human intestinal absorption of a variety of substances are rapidly gaining international popularity and the quality, quantity and variety of information in databases available to develop these models is increasing such that such modeling methods are becoming an accepted and regular fixture in many current pharmaceutical development projects.

SUMMARY

Intestinal absorption encompasses many highly significant and important processes both in health and disease. As our understanding of normal intestinal function improves so will our ability to recognize and potentially treat or correct abnormal intestinal function. Advances being made in genomics, proteomics, computational modeling, and nanotechnology are certain to have major impacts toward advancing both our knowledge of human intestinal function and the quality of life of those with conditions where intestinal function is abnormal in some manner. The sciences of gastroenterology and toxicology will be important contributors in this advancement.

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CHAPTER 13

Classes of Compounds with GI Tract Toxicity

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INTRODUCTION

The gastrointestinal tract, which accounts for one of the largest organ systems in the body, is typically associated with absorption of nutrients and excretion of excess waste derived from food consumption. However, the GI tract also acts as the main point of entry for orally administered pharmaceutical products and other compounds which may be present in food or water. Within this organ system, compounds may undergo initial steps in metabolism via action of commensal bacteria in the gut, endogenous Phase I (e.g., cytochrome P450 enzymes) and Phase II enzymes (e.g., glutathione S-transferases), and transport proteins within the membrane of the GI tract, and potentially be absorbed and transported into the bloodstream. Therefore, the potential for unintended toxicities may arise if the GI tract is damaged or the membrane barrier function is altered or impaired. Some compounds, such as opiates, have direct effects upon the GI tract due to receptor binding, while other compounds may elicit adverse events related to general physicochemical characteristics (such as pH, solubility, and bioavailability). The GI tract has defense mechanisms to protect the body from toxicants, but these are occasionally breached, resulting in compound-induced toxicity.

Numerous compounds are associated with gastrointestinal toxicities; here, we provide background information on specific drugs and drug classes which are known to be toxic to the GI tract to form a basis for further inquiry by the reader. The selected compounds, which include Rotashield®, Lotronex®, and Slow-K potassium chloride tablets, ethanol, bisphosphonates, opiates, and non-steroidal anti-inflammatory drugs, are not intended to fully cover all drugs known to elicit gastrointestinal toxicities, but to highlight those that may have GI-specific mechanisms of toxicity.

ROTASHIELD®

Clinical Use of Rotashield®

In 1998, the United States Food and Drug Administration (FDA) licensed vaccine known Rotashield® which was marketed to prevent rotavirus in infants and young children. Rotavirus is the leading cause of viral gastroenteritis (severe vomiting and diarrhea) in children between the ages of 3 months and 5 years worldwide. The exact mechanism of action exerted by natural rotavirus infection is incompletely understood. However, this virus is suggested to be primarily transmitted by the fecal-oral route, and can last on human hands for hours, days on inanimate surfaces, and resist common disinfectants (Bernstein, 2009). This infection is characterized as a collection of non-enveloped segmented double-stranded RNA viruses from the Reoviridae family. Most of the human rotavirus infections are the result of exposure to group A viruses (Pasetti et al., 2011). The outermost layer of the viral nucleocapsid of rotaviruses is composed of two viral surface proteins, VP4 (protease sensitive, P serotypes) and VP7 (glycoprotein G serotypes). Administration of the rotavirus vaccine, Rotashield™, induces antibodies which target the neutralization of these serotypes in rotavirus strains (Payne et al., 2016). In addition, use of Rotashield® was advantageous based on its capability to replicate less efficiently in the human intestines when compared to natural human rotaviruses and protect against the challenge, controlled strain.

Mechanism of Action

Rotashield® is a human-rhesus rotavirus tetravalent oral vaccine which is suggested to mimic the immunologic responses stimulated by rotavirus. The four live viruses which make up this vaccine include a rhesus rotavirus serotype G3 (which cross-reacts with human G3) and three rhesus-human reassortant viruses encoding VP7 for human serotypes G1, G2, and G4 (Bernstein, 2009; Dennehy, 2008; Hochwald & Kivela, 1999; Wang et al., 2015). Collectively, these serotypes activate the production of IgG and IgA serum antibodies in the intestines which neutralize these viruses and induce a local immune response. Infants are administered this vaccine in a 3-dose regimen at 2, 4, and 6 months of age (Hoshino & Kapikian, 2000).

Mechanism of Toxicity

Use of Rotashield® was suspended 9 months post-licensure in the United States after 15 cases of intussusception in infants were reported to the Vaccine Adverse Events Reporting System (VAERS), which is jointly sponsored by the FDA and the U.S. Centers for Disease Control and Prevention (CDC) (Bernstein, 2009). Infants > 90 days old accounted for 80% of intussusception-associated cases as a result of first dose administration of Rotashield®. Intussusception is a rare, but serious condition in which a proximal segment of the intestines (intussusceptum) fold into the lumen of the

adjacent distal segment (intussusciptions) (Bernstein, 2009). The “bowel telescoping” effect of the intussusception results in a blockage of food and fluid from efficiently passing through the intestines. Additionally, intussusception restricts venous return and arterial blood flow to the intestines, which can lead to edema, gastrointestinal perforation (bowel obstruction), infection, and structural abnormalities such as polyps or tumors (Midthun & Kapikian, 1996; Offit, 2002). If not treated early, the onset of intussusception may cause death (Murphy et al., 2001). The greatest risk of an infant developing intussusception occurs 3–7 days following administration of Rotashield® vaccine (Murphy et al., 2001). Though a strong relationship between Rotashield® administration and intussusception has been suggested, the exact mechanism of toxicity is unknown. However, it is speculated that Rotashield® replicates in the small intestinal mucosal surface thereby inducing intussusception (Offit, 2002, 2006). Currently, no plan has been implemented to reintroduce Rotashield® vaccine into either developing or developed countries.

LOTRONEX®

Clinical Use of Lotronex®

The drug product, Lotronex®, is a potent selective 5-hydroxytryptamine 3 (5-HT₃) serotonin receptor antagonist approved in February 2000 to treat women with severe diarrhea-predominant irritable bowel syndrome (IBS) (Ervin & Mangel, 2013; Ford & Vandvik, 2012; Miller et al., 2003; Nee et al., 2015; Vanuytsel et al., 2014). Most severe symptoms of IBS are characterized by frequent and severe abdominal pain/discomfort, frequent bowel urgency or fecal incontinence, and restriction of daily activities due to IBS. Lotronex® is typically given via oral route of administration as a tablet to women with chronic IBS symptoms lasting more than 6 months who have not adequately responded to other conventional treatment options and do not contain any anatomic or biochemical gastrointestinal tract-related disorders (Ervin & Mangel, 2013; Mayer & Bradesi, 2003).

The active ingredient of Lotronex® is alosetron hydrochloride and chemically designated as 2,3,4,5-tetrahydro-5-methyl-2-[(5-methyl-1H-imidazol-4-yl)methyl]-1H-pyrido[4,3-b]indol-1-one, monohydrochloride. Structurally, alosetron is an achiral compound (as shown in Figure 13.1) and has a molecular weight of 330.8 g/mol.

Mechanism of Action

Structurally, 5-HT₃ serotonin receptors are ligand-gated channels extensively distributed on enteric neurons in the human gastrointestinal tract, in addition to peripheral and central locations (Shah et al., 2012; Vanuytsel et al., 2014). Upon activation and subsequent neuronal depolarization of 5-HT₃ serotonin receptors, pathophysiological-associated IBS effects including increased gastrointestinal

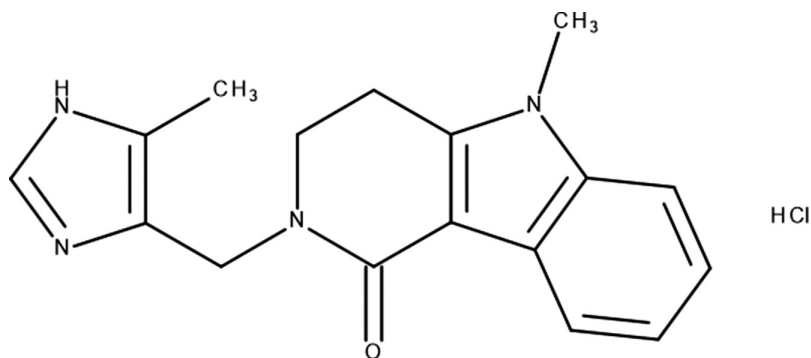


Figure 13.1 Structure of Alosetron.

secretions (e.g., loose or watery stool, and frequent bowel movements), increase sensation of visceral pain, and accelerated colonic transit result (Miller et al., 2003).

Administration of alosetron, is suggested to bind to and prevent the activation of 5-HT₃ serotonin receptors located in the intestines in a slow, reversible manner (Chang et al., 2006; Chang et al., 2010; Mayer & Bradesi, 2003; Miller et al., 2003; Nee et al., 2015). As a result, alosetron-mediated inhibition of 5-HT₃ serotonin receptor activation leads to a decrease in serotonin signaling. Additionally, administration of alosetron reduces pain sensitivity due to rectal distension and progressively slows colonic transit which increases the amount of water absorbed and decreases the moisture and volume of remaining waste products.

Mechanism of Toxicity

In November 2000, alosetron was voluntary withdrawn from the U.S. market after approximately 100 cases of ischemic colitis were reported in association with this drug (Chang et al., 2010; Gallo-Torres et al., 2006; Lewis, 2010). Ischemic colitis is the most frequently encountered form of ischemic injury to the gastrointestinal tract which involves such insults as reversible, transient segmented colitis, acute transmural necrosis (gangrenous colitis), chronic colitis with structure formation, and fulminant colitis (Lewis, 2010). In general, ischemic colitis is associated with an acute onset of lower abdominal pain, cramping, bloody diarrhea, or hematochezia. Several hypotheses have been proposed in support of alosetron-associated ischemic colitis. One proposed theory suggests that alosetron-induced blockage of 5-HT₃ receptors causes serotonin (e.g., platelets, enterochromaffin cells, endothelial cells, or from adrenergic neurons) to bind and activate, in excess, other 5HT receptors including, 5HT₁ and 5HT₂ receptors (Beck, 2001). As a result, cross-talk mechanisms between serotonin receptors may occur, and indirectly mediate vasoconstriction (Beck, 2001). Another proposal has suggested that alosetron may induce serious complications of constipation including fecal impaction, intestinal/ileus obstruction, toxic megacolon, and intestinal perforation. In the most severe

cases, alosetron-associated constipation may increase intraluminal pressure with a subsequent reduction in colon blood flow and a generation of blood shunting from the mucosa to the serosa; hence, patients are predisposed to mucosal ischemia.

Although alosetron was withdrawn from the U.S. in 2000, this drug was reintroduced 2 years later with a very stringent indication and Risk Management Plan. Under these new guidelines, only women who meet all of the following criteria are allowed to take alosetron: (1) experienced severe IBS-associated diarrhea (characterized by frequent and severe abdominal pain or discomfort, increased frequency in bowel movements or fecal incontinency, and/or restriction to daily as due to) which have lasted for at least 6 months; (2) do not possess any anatomic or biochemical abnormalities of the gastrointestinal tract which may increase their risk for developing ischemic colitis or serious complications with constipation; and (3) failed to adequately respond to conventional therapy (Lewis, 2010).

SLOW-K POTASSIUM CHLORIDE TABLETS

Clinical Use of Slow-K Potassium Chloride (KCl) Tablets

Slow-K is an extended-release wax core oral tablet used to treat patients suffering from potassium depletion. Potassium ion (K^+) is an essential intracellular cation for most body tissues. This ion is vital to maintain intracellular tonicity and normal renal function, transmission of nerve impulses, and contraction of smooth, cardiac, and skeletal muscles. The intracellular concentration of K^+ is approximately 150–160 mEq/L (Ohtsu, 1956; Worth, 1988). Normally, plasma potassium concentrations in adult humans range from 3.5 to 5 mEq/L (Worth, 1988). An active ion transport system maintains this gradient across the plasma membrane.

K^+ depletion may result when the rate of potassium intake is less than the rate of potassium loss from the gastrointestinal tract and/or renal excretion. K^+ depletion is generally a slow process as a consequence of primary or secondary hyperaldosteronism, diabetic ketoacidosis, severe diarrhea, inadequate replacement of potassium from prolonged parenteral nutrition, or prolonged treatment of oral diuretics.

Administration of supplemental KCl acts to replenish potassium deficiency. As a result, use of this drug aids in the restoration normal renal function, conduction of nerve impulses in the heart, brain and skeletal muscle, contraction of muscles, acid-base balance, carbohydrate metabolism, and gastric secretion (Hillman, 1966; Khait, 1966; Marinis & Muirhead, 1947).

Mechanism of Toxicity

The most common adverse effects to the gastrointestinal tract as a result of KCl usage are the production of esophageal, gastric, small bowel ulcerations as well as stenotic lesions (Aselton & Jick, 1983; Buchan & Houston, 1965; Farquharson-Roberts et al., 1975; Pietro & Davidson, 1990). In a 1982 gastrointestinal study,

McMahon et al., (1982) compared a wax-matrix slow-release formulation of KCl to enteric coated KCl preparations. In this three-part study, human volunteers were subjected to one of the following procedures: (i) oral administration of wax-matrix KCl preparation (Slow-K) or microencapsulated KCl (Micro-K), three times a day (2.4 g KCl/dose) for seven days in combination with the anticholinergic medication, glycopyrrolate (2 mg/dose, three times per day); (ii) oral administration of oral administration of Slow-K or Micro-K, three times a day for seven days at 2.4 g KCl/dose; and (iii) oral administration of oral administration of oral administration of Slow-K or Micro-K, three times a day for seven days at 0.6 g KCl/dose with glycopyrrolate (2 mg/dose, three times per day). In all studies, while both formulations induced mucosal lesions, gastric ulcers, upper gastrointestinal bleeding, edema, multiple gastric erosion, and inflammatory lesions these endoscopic findings were more severe following administration of Slow-K. Furthermore, the endoscopic lesions were more prevalent in the prepyloric area, entire antrum of the stomach, distal esophagus, and duodenal bulb. Co-administration of glycopyrrolate, which slows the gastrointestinal transit time, exacerbate KCl-induced mucosal lesions. In addition, concentrated localization of potassium salt was observed to induce esophageal ulcers (Kikendall, 1991; Yap et al., 1993). Based on these results, Slow-K-induced endoscopic lesions were suggested to form as a result of high localized concentrations of KCl in the gastrointestinal tract (McMahon et al., 1982, 1984; Parfitt & Driman, 2007). Moreover, oral administration of Slow-K was proposed as extreme concern in elderly patients, some hypokalemic patients, immobilized individuals, postoperative patients, diabetic patients with neuropathy, and patients receiving anticholinergic treatment. Oral administration of Slow-K should be limited in these individuals since their gastrointestinal transit is further delayed (Kikendall, 1991; McMahon et al., 1982).

Currently, it is estimated that oral administration of KCl as low as 31 mg/kg bw/day causes gastrointestinal irritant effects. While Slow-K is widely considered safe for use, it is strongly encouraged that a patient takes this drug with an adequate amount of water (8 ounces/240 mL). If by chance an individual develops KCl-induced injury due to oral administration of Slow-K, a patient should switch to microencapsulated KCl preparations. Use of microencapsulated KCl formulations are suggested as an alternative to Slow-K because of its ability to cover a wide surface area of the stomach, thereby preventing the potential erosive effect of K^+ ions with the mucosal lining (McMahon et al., 1982).

ETHANOL

Clinical Use of Ethanol

Ethanol (CAS# 64-17-5), commonly known as “alcohol,” is composed of a hydroxyl group attached to an ethyl group. It is historically one of the most widely used drugs of abuse deemed socially appropriate in many societies worldwide. Ethanol may also be used for industrial purposes as solvents, antifreeze agents,

brake fluids, paints, resins, and fuels, and medicinal purposes as a drug solvent, topical antimicrobial, nerve block, or treatment for methanol and ethylene glycol poisoning. Denatured ethanol, which is rendered unsafe to drink by the addition of approved denaturants per intended use, is often utilized in various cosmetic preparations (Sivilotti, 2004).

Mechanisms of Toxicity

The gastrointestinal (GI) tract plays an outsized role in regards to the effects and toxicities elicited by ethanol (especially in the form of alcohol). Upper GI tissues directly exposed to alcohol may undergo metabolic and functional changes, resulting in a variety of toxicities including increased risk of esophageal cancer, impaired function of the esophageal sphincter, altered gastric acid secretion in the stomach, acute GI bleeding from the stomach or small intestine, effects on peristalsis, and altered absorption of nutrients and toxins present in the GI tract (Bode & Bode, 1997).

Alcohol is absorbed into the bloodstream within the GI tract, which is a common site of alcohol-induced toxicity. Ethanol is primarily metabolized via oxidation by three major mechanisms including the alcohol dehydrogenase pathway, cytochrome P450 pathway, and catalase- H_2O_2 pathway. About 90% of ingested alcohol is oxidized in the liver, and the remainder undergoes extrahepatic oxidation in the gastrointestinal tract by resident bacteria, the kidneys, and excretion of bodily fluids and exhalation. The primary metabolite, acetaldehyde, is a known toxicant and carcinogen capable of forming DNA adducts but may be further metabolized into acetate in the liver prior to excretion. Acetaldehyde metabolites formed in the GI tract, however, may be detected in the saliva and breath of intoxicated humans, and are suggested to play a role in GI carcinogenesis, especially in the esophagus, oral cavity, pharynx, and larynx (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2010; IARC Monograph on Consumption of Alcoholic Beverages, 2012).

Esophageal Toxicity

Tissues of the upper GI tract are exposed to nearly undiluted alcohol prior to reaching the stomach, and at increased acute risk of mucosal damage at doses exceeding two ounces (roughly equivalent to four standard alcoholic beverages in one sitting, typically defined as “heavy” drinking). Chronic alcohol abuse may result in hypertrophy of the parotid glands (primary salivary glands), dental problems, glossitis (tongue inflammation), stomatitis (mouth inflammation), abnormal esophageal motility, and esophagitis, which may present with or without concurrent erosions. Furthermore, chronic alcoholics may develop Barrett’s esophagus, a disorder characterized by epithelial dysfunction and abnormal acid secretion used as an indicator for increased risk of developing esophageal cancer. Alcoholics may also develop Mallory-Weiss syndrome, identified by excessive bleeding resulting from mucosal tears at the gastroesophageal junction related to repetitive vomiting (Bode & Bode, 1997).

Toxicity in the Stomach and Small Intestine

Ethanol may elicit a variety of stomach- and intestine-specific toxicities, including mucosal damage, altered gastric acid secretion, changes to the intestinal microbiome, altered motility and absorption of nutrients from food, and possible carcinogenesis, which will be described below. Alcohol-induced mucosal damage varies between short-term and chronic use, and exposure level. Concentrations exceeding 10% alcohol are known to damage the mucosal barrier and induce focal areas of mucosal hyperaemia, edema, epithelial necrosis, and mucosal hemorrhage, while chronic use may result in altered GI microcirculation and additional advanced damage to the mucosal barrier (Bode & Bode, 1997; Bode et al., 1996). Animal and human studies have suggested that endogenous prostaglandins, including PGE₂ and PGI₂, play a key role in maintaining gastric mucosa structure and function. Prostaglandins, which are eicosanoids derived from 20-C chain unsaturated fatty acids, aid in maintaining GI barrier function, stimulate bicarbonate release (pH maintenance) and gastric mucosal blood flow, and play an underlying role in tissue repair of damaged GI epithelium (Hawkey & Rampton, 1985). In a study performed by Bode et al., subjects acutely exposed to 12.5% alcohol exhibited reduced PGE₂ production, although PGF_{2α} and 6-keto PGF_{2α} levels did not change. Further study in a cohort of chronic alcohol users determined that alcohol may reduce synthesis of PGE₂ and PGF_{2α} which may lead to increased risk of mucosal dysfunction (Bode et al., 1996).

Low concentrations of pure ethanol (<5%) stimulate gastric acid secretion, while higher concentrations elicit limited to inhibitory effects. Exposure to lower concentrations of ethanol in alcoholic beverages (e.g., in beer and wine) strongly stimulates gastric acid production and gastrin secretion compared to high alcohol concentration substances (e.g., in whiskey and other liquors), although a direct correlation between gastric acid secretion and gastrin production has not been established (Chari et al., 1993). Several mechanisms of action have been proposed regarding alcohol-mediated changes in gastric acid secretion, which may be direct or systemic. Alcohol (at low concentrations) may have topical and systemic effects mediated by cholinergic nerves or histamine release from the gastric mucosa. Chronic users exhibit atrophy of the gastric mucosa and reduced gastric secretory capacity, which may alter the gut microbiome (Bode & Bode, 1997).

Alcohol has been shown to induce gut dysbiosis (microbial imbalance) *in vivo* and in humans; additionally, intestinal dysbiosis and translocation of pathogenic bacteria in the GI tract have been associated with pathogenesis of alcoholic liver disease (Cresci, 2015). The microbiome, composed of trillions of microorganisms sensitive to environmental and dietary changes, forms a symbiotic relationship with the GI tract, aiding in extraction of nutrients from food and barrier protection to pathogenic organisms (Engen et al., 2015). Hartmann et al. (2015) suggested that alcoholics, alcoholics with liver cirrhosis, and moderate drinkers all had observed changes in the quantity and ratio of microbial species present in the gut, with lower levels of beneficial commensal bacteria such as Lachnospiraceae, Ruminococcaceae, and Clostridiales Family XIV *Incertae sedis* and increased levels of potentially pathogenic bacteria

such as Enterobacteriaceae, Streptococcaceae, and Veillonellaceae, especially in cirrhotic patients. Upon cessation of alcohol use, eubiosis (microbial balance) can be at least partially restored (Hartmann et al., 2015).

Alcohol use may also impact gastric and intestinal motility dependent upon the beverage consumed and alcohol concentration, with lower concentrations (beer and wine) accelerating and higher concentrations (>15%; e.g., liquor) delaying gastric motility and emptying (Bode & Bode, 1997; Pfeiffer et al., 1992; Stermer, 2002). Alcohol also decreases wave motility function in the small intestine, which reduces time for additional digestive processes. A study comparing alcoholics consuming an average of 191.1 g alcohol/day, “social drinkers,” and abstainers determined that the orocecal transit time was significantly longer in alcoholics compared to other subject groups, providing further evidence of the effects alcohol may have upon small intestine contraction and vagal function (Addolorato et al., 1997).

Chronic alcohol abusers often develop intestinal disorders including diarrhea, altered intestinal permeability, and malabsorption related to both altered digestion and absorption. Diarrhea may be related to reduced enzymatic activity in intestinal mucosa and increased intestinal permeability resulting in an influx of water and solutes into the intestinal lumen (Bujanda, 2000). Enhanced permeability may allow for passage of endotoxin and other bacterial toxins from the intestinal lumen into the bloodstream, potentially resulting in inflammation and development of subsequent liver toxicity and other toxicities (Bode & Bode, 1997). Transport and absorption of nutrients are also significantly altered in alcoholics, despite a lack of obvious histological or macroscopic changes to the intestines. Water, sodium, thiamine, vitamin B12, calcium, iron, and zinc, and basic nutritional groups including carbohydrates, lipids, and proteins have all been shown to exhibit altered uptake in the intestines of alcoholics. A study assessing absorption of carbohydrates, lipids, and proteins in the duodenum showed a 45, 40, and 81% decrease in absorption of these macromolecules, respectively, in alcoholic subjects compared to non-alcoholics (Bode & Bode, 1997; Bujanda, 2000; Pfeiffer et al., 1992).

Carcinogenicity

IARC assessed the potential for carcinogenicity associated with intake of alcoholic beverages, and determined alcoholic beverages, ethanol, and acetaldehyde to be carcinogenic to humans (Group 1 compounds) capable of causing cancers of the oral cavity, pharynx, larynx, esophagus, colorectum, liver, and breasts of females (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2010). Potential mechanisms of action of carcinogenesis associated with chronic alcohol use include production of reactive oxygen species (ROS), acetaldehyde, genetic polymorphisms of genes associated with ethanol metabolism, and suppressed immune surveillance (Ratna & Mandrekar, 2017). Countless studies and epidemiological assessments have been carried out regarding alcohol-induced carcinogenicity, generally concluding that consumption increases risk of carcinogenesis in a dose- and consumption-specific manner (Badger et al., 2003; Bujanda, 2000; Everatt et al., 2012; Grønþæk et al., 1998; Scoccianti et al., 2016; Seitz et al., 2005).

BISPHOSPHONATES

Clinical Use of Bisphosphonates

Bisphosphonates are metabolically stable pyrophosphate analogs primarily used in the treatment of osteoporosis, bone-related malignancies, and other bone disorders, such as Paget’s disease. These compounds bind divalent cations (Ca²⁺) from circulation and are endocytosed by osteoclasts, osteoblasts, macrophage, circulating monocytes, epithelial and endothelial cells, and some neoplastic cells (Papapetrou, 2009). Two phosphate groups and a hydroxyl group structurally present in all bisphosphonates enable binding to hydroxyapatite, the principal mineral component of bone that provides mechanical and load-bearing strength (Drake et al., 2008). The primary mechanism of action associated with nitrogen-containing bisphosphonates is through induction of apoptosis in osteoclasts via inhibition of farnesyl diphosphate (FPP) synthase and subsequent prenylation of GTPases, which control numerous cellular processes (Papapetrou, 2009).

The bisphosphonate class is primarily divided into two major groups based upon presence of nitrogen within the R₂ alkyl group, which may impact the potency and expected toxicity of the drug (Bock & Felsenberg, 2008). All bisphosphonates share the general structure presented below in Figure 13.2. Table 13.1 provides compound names, CAS identifiers, and structures of common bisphosphonates.

The structure of the R₂ group plays a key role in modulating bone adsorption and binding affinity to FPP synthase, which is generally proportional to

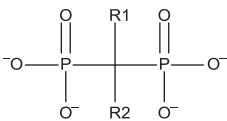


Figure 13.2 General structural backbone of bisphosphonates.

Table 13.1 Summary of Selected Bisphosphonates

Compound Class	Compound	Structure
Non-nitrogen containing bisphosphonates	Clodronate	
	Etidronate	

(Continued)

Table 13.1 (Continued) Summary of Selected Bisphosphates

Compound Class	Compound	Structure
Nitrogen-containing bisphosphonates	Alendronate (CAS# 66376-36-1)	
	Risedronate (CAS# 105462-24-6)	
	Ibandronate (CAS# 114084-78-5)	
	Zoledronic acid (CAS# 118072-93-8)	
	Pamidronate (CAS# 40391-99-9)	

antiresorptive activity. Second-generation bisphosphonates containing free amino groups include pamidronate and alendronate, while newer, more potent compounds such as ibandronate, risedronate, and zoledronate contain more highly branched or heterocyclic nitrogen groups (Bock & Felsenberg, 2008). Nancollas et al. (2006) assessed binding activities of six first and second-generation bisphosphonates to hydroxyapatite using an *in vitro* model of hydroxyapatite crystal growth; results suggested that R2 side chains may alter physicochemical properties associated with hydroxyapatite crystals which enhance bisphosphonate uptake, encourage biopersistence, enhance bone mineral binding affinity, and improve antiresorptive potency.

Despite structural differences, bisphosphonates typically exhibit similar pharmacokinetic properties including poor intestinal absorption, short plasma half-life, extensive drug retention in bone, and elimination via the urine. Approximately 40%–60% of a dose is absorbed by the skeleton, while the rest is excreted unchanged via the urine. Terminal half-life may vary, and the compounds are expected to accumulate, which may contribute to long-term reduction in bone turnover (Bock & Felsenberg, 2008). However, structural properties may significantly impact attachment to bone and subsequent duration of activity as well as inhibition of FPP synthase, which together modulate bisphosphonate activity (Russell, 2006).

Mechanisms of Toxicity

Nitrogen-containing oral bisphosphonates including alendronate, risedronate, and ibandronate, have been associated with upper gastrointestinal adverse effects that have not been observed following intravenous administration of these compounds or other bisphosphonates (Papapetrou, 2009).

Bisphosphonates are usually associated with upper gastrointestinal tract toxicity due to mucosal irritation. Although nausea, vomiting, epigastric pain, and dyspepsia may occur, the primary toxicity associated with bisphosphonates are esophagitis and esophageal erosions or ulcerations. Pill esophagitis, which may occur upon prolonged contact of a pill with tissue lining the esophagus, has been observed during post-marketing of alendronate, especially in patients who ingested the pill with little or no water, laid down after ingestion, continued taking doses after signs and symptoms of esophageal toxicity, or had pre-existing esophageal conditions (Graham, 2002; Papapetrou, 2009).

Pamidronate, a second-generation bisphosphonate, elicited erosive esophagitis and reduced gastrointestinal tolerability at doses exceeding 150 mg/day during clinical testing, and was not approved for oral use by the FDA (Graham, 2002). The majority of gastrointestinal toxicities have been observed with second- and third-generation bisphosphonates alendronate and risedronate, which did not appear during clinical trials. Upper gastrointestinal adverse events, including esophageal ulceration, esophagitis, and erosive esophagitis, were the most commonly reported AEs during post-marketing assessment of alendronate, and related to patient non-compliance in nearly two-thirds of patients aware of administration information supplied with the drug, suggesting that toxicity may be administration instead of drug-related (Strampel et al., 2007). Further testing was completed in several clinical

trials, including the Fracture Intervention Trial, a randomized, double-blind, placebo-controlled study of 6459 postmenopausal women with (2027 subjects) and without vertebral fractures (4432 subjects) followed for gastrointestinal toxicities over 3 and 4.5 years, respectively. Rates of discontinuation (3.2% vs 2.7%) and overall incidence of adverse effects (47.5% vs 46.2%) were nearly equal between alendronate and placebo treated subjects as assessed. Results suggested that the treatment with alendronate (5 mg/day the first 2 years, increased to 10 mg/day for remainder of study) did not elicit statistically relevant upper gastrointestinal toxicities, even in women >75 years old with previous upper GI tract disease or concomitant NSAID use expected to exhibit increased risk for toxicity (Bauer et al., 2000; Graham, 2002). A meta-analysis of nine randomized placebo-controlled trials assessing risedronate in 10000 patients did not find evidence of drug-induced upper GI toxicity, considering that 29.6% and 29.8% of respective placebo and drug-treated groups exhibited GI toxicities with no significant differences in observed inflammation or esophageal, gastric, or duodenal ulceration between patient groups (Strampel et al., 2007).

It is of note that non-nitrogen containing bisphosphonates, including clodronate and etidronate, are less potent and have not been associated with upper gastrointestinal toxicities during clinical trials or in the post-marketing experience (Graham, 2002).

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

Clinical Use of Non-Steroidal Anti-Inflammatory Drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely prescribed and utilized drug classes in the world for treatment of conditions including inflammation, acute pain, fever, and arthritis. The primary pharmacological target of NSAIDs, including acetylsalicylic acid (“aspirin”), is the cyclooxygenase isoenzymes, COX-1 and COX-2, which are involved in the synthesis of prostanoids (prostaglandins and thromboxanes) from free arachadonic acid (Bruno et al., 2014; Martinez-Gonzalez & Badimon, 2007). Prostanoids, especially PGE₂ and PGI₂, are pro-inflammatory, enhancing vascular permeability, leukocyte infiltration, pyresis, and pain. These chemical messengers also play a role in maintaining renal function, cardiovascular homeostasis, and inhibition of platelet activation and recruitment (Smyth et al., 2009). Additional information regarding COX isoenzymes may be found in [Table 13.2](#).

Due to the ubiquitous nature of COX isoenzymes in a majority of bodily tissues, especially within the GI tract and cardiovascular system, it is unsurprising that NSAID-mediated inhibition may also elicit serious or fatal adverse effects in some patients that vary based upon the physicochemical properties and mechanisms of action associated with specific NSAID classes. For instance, acidic NSAIDs typically exhibit a high degree of protein binding associated with enhanced accumulation and persistence at sites of inflammation, while non-acidic NSAIDs are more likely to distribute throughout the body (Brune & Patrignani, 2015). NSAIDs are primarily divided into four classes, which are further described below:

Table 13.2 Summary of COX-1 and COX-2 Tissue Expression and Function

Isoenzyme	Expression	Function
COX-1	Constitutively expressed “housekeeping” enzyme typically identified in the gastrointestinal tract, platelets, and kidneys	Maintaining gastrointestinal mucosal integrity via production of gastroprotective prostaglandins PGE2 and prostacyclin (PGI2) Stimulating platelet aggregation via induction of thromboxane synthesis Regulating homeostatic renal function via tubular PGE2 and vascular PGI2 (Hörl, 2010; Knijff-Dutmer et al., 2002; Kwiecien et al., 2015; Wolfe et al., 1999)
COX-2	Generally absent in most tissues under normal physiologic conditions, but constitutively expressed at detectable levels in CNS, kidney, and blood vessels and inducible in macrophage, fibroblasts, synovial, and endothelial cells upon exposure to inflammatory or mitogenic stimuli such as interleukin-1 β (IL-1 β), interleukin 15 (IL-15), tumor necrosis factor α (TNF- α), and lipopolysaccharides (LPS) (Bingham et al., 2006; Hayashi et al., 2014; Wolfe et al., 1999)	Regulates renal function Present in reproductive tissues and important for ovulation and onset of labor at end of pregnancy Promotes healing at sites of gastromucosal damage via production of prostaglandins associated with mucosal function Supports production of PGI2 associated with atheroprotection Induces production of PGI2 in vascular endothelial and smooth muscle cells which inhibits platelet activity (Smyth et al., 2009; Zarghi & Arfaei, 2011)

Traditional or Non-Selective NSAIDs

Traditional, or non-selective (NS)-NSAIDs inhibit both COX-1 and COX-2 activity, thus limiting the gastroprotective effects of COX-1-derived prostaglandins in addition to COX-2-mediated pro-inflammatory effects such as vascular constriction and permeability, pain, and pyresis. In clinical practice, NS-NSAIDs are commonly associated with inducing gastrointestinal toxicities such as bleeding, gastroduodenal mucosal injury, lesions, and ulcers (Kwiecien et al., 2015; Sostres et al., 2013; Wolfe et al., 1999).

Cox-2 Selective NSAIDs

COX-2 inhibitors are designed to be highly selective for the COX-2 binding site, which, due to substitution of several key amino acids, is structurally and chemically distinct from COX-1 (Zarghi and Arfaei, 2011). Clinical studies suggest that these compounds are markedly less likely to result in upper gastrointestinal toxicities, but may carry increased risk of cardiovascular toxicity (Bhala et al., 2013; Day & Graham, 2013; Sostres et al., 2013; Wolfe et al., 1999).

Cox-Inhibiting Nitric Oxide Donators

Cox-inhibiting nitric oxide donators (CINODs) are next-generation non-selective NSAIDs with attached nitric oxide (NO) moieties. Upon release, NO may support maintenance of gastrointestinal mucus via reduction of leukocyte-endothelial adhesion in the gastric vasculature, enhanced gastric mucosal blood flow, and increased mucus and bicarbonate secretion and also act as a homeostatic regulator of the vasculature (Kwiecien et al., 2015; Schnitzer et al., 2010). *In vivo* studies suggest that CINODs induce fewer gastrointestinal injuries to the esophagus, gastric mucosa, and stomach compared to parent NSAIDs (Fiorucci et al., 2007; Kwiecien et al., 2015).

Hydrogen Sulfide NSAIDs

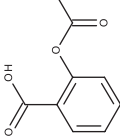
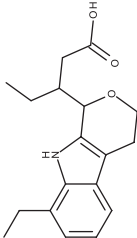
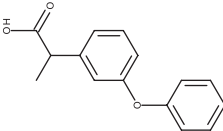
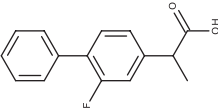
H₂S NSAIDs, which contain hydrogen sulfide moieties, are still under investigation in the preclinical phase, and clinical trials have yet to start. Significantly reduced gastric damage and endothelial leukocyte adherence, an early marker of inflammation, were reported in a small scale *in vivo* study comparing H₂S-releasing diclofenac and indomethacin to respective parent compounds, suggesting that presence of the H₂S moiety may reduce gastrointestinal toxicity of NSAIDs (Sostres et al., 2013; Wallace, 2007).

Table 13.3 below provides a summary of NSAID compound names, CAS identifiers, and structures.

Mechanisms of Toxicity

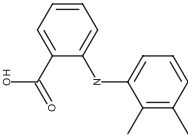
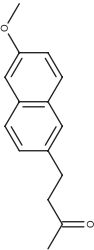
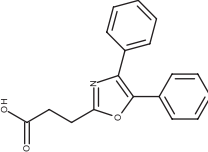
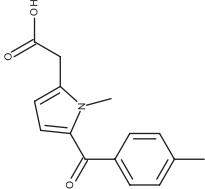
Gastrointestinal toxicities are the most prevalent adverse effects associated with NSAIDs and may vary in severity. Approximately 1%–2% of patients administered NSAIDs develop serious GI complications during treatment that may require hospitalization (Sostres et al., 2013). NSAID-related GI toxicities are often associated to inhibition of COX-1, which is primarily responsible for maintenance of the gastric mucosa and bicarbonate secretion; however, COX-2 inhibition may result in delayed healing of pre-existing gastric ulcers (Brune & Patrignani, 2015; Bruno et al., 2014). Suppression of prostaglandin synthesis significantly impacts repair of the gastric mucosa, microcirculation and mucosal blood flow, epithelial cell proliferation and repair, and mucosal immune cell function (Wallace, 2000). Observed dose-dependent toxicities may also be attributed to the pharmacokinetics and physicochemical properties of individual NSAIDs; compounds with long plasma half-lives, slow release formulations, or acidic properties are more likely to result in toxicities. Acidic compounds, which are nonionized at the standard gastric pH and freely lipid-soluble, may directly damage gastric epithelium by penetrating and accumulating in neutral pH mucosal cells following intercellular conversion to a more toxic ionized form (Bruno et al., 2014; Lazzaroni & Bianchi Porro, 2004). Risk of gastrointestinal toxicities vary by type and dose of NSAID administered, older age, prior ulcer, concomitant use of other NSAIDs, anticoagulants, antiplatelet agents, corticosteroids, or selective serotonin reuptake inhibitors (SSRIs), severe illness, and active *Helicobacter pylori*

Table 13.3 Summary of Selected Bisphosphonates

Compound Class	Compound	Structure
Non-Selective NSAIDs (COX-1 and COX-2 inhibitors)	Acetylsalicylic Acid; Aspirin (CAS# 50-78-2) ^a	
	Etodolac (CAS# 41340-25-4) ^a	
	Fenoprofen (CAS# 29679-58-1) ^a	
	Flurbiprofen (CAS# 5104-49-4) ^a	

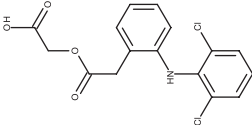
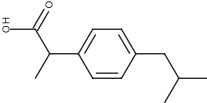
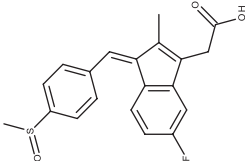
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Table 13.3 (Continued) Summary of Selected Bisphosphonates

Compound Class	Compound	Structure
Non-Selective NSAIDs (COX-1 and COX-2 inhibitors) (Continued)	Mefenamic Acid (CAS# 200-513-1) ^a	
	Nabumetone (CAS# 42924-53-8) ^a	
	Oxaprozin (CAS# 21256-18-8) ^a	
	Tolmetin (CAS# 64490-92-2) ^a	

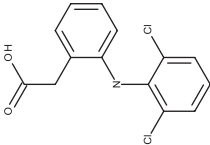
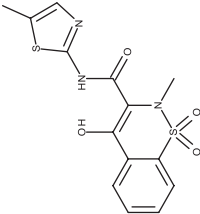
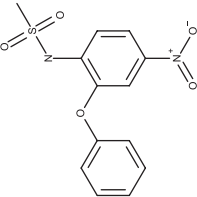
(Continued)

Table 13.3 (Continued) Summary of Selected Bisphosphates

Compound Class	Compound	Structure
Non-Selective NSAIDs (COX-1 and COX-2 inhibitors) (Continued)	Acetclofenac (CAS# 89796-99-6) ^a	
	Ibuprofen (CAS# 15687-27-1) ^a	
	Sulindac (CAS# 38194-50-2) ^a	

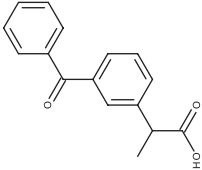
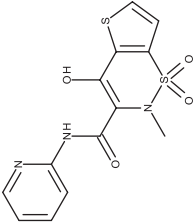
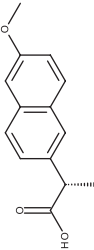
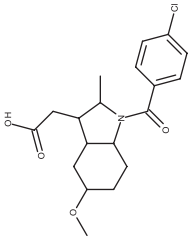
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Table 13.3 (Continued) Summary of Selected Bisphosphonates

Compound Class	Compound	Structure
Non-Selective NSAIDs (COX-1 and COX-2 inhibitors) (Continued)	Diclofenac (CAS# 15307-86-5) ^a	
	Meloxicam (CAS# 71125-38-7) ^a	
	Nimesulide (CAS# 51803-78-2) ^a	

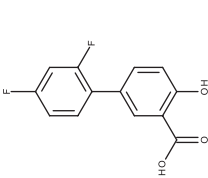
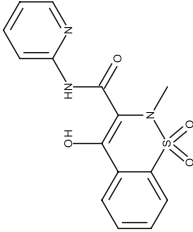
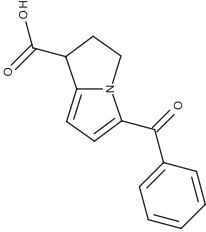
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Table 13.3 (Continued) Summary of Selected Bisphosphates

Compound Class	Compound	Structure
Non-Selective NSAIDs (COX-1 and COX-2 inhibitors) (Continued)	Ketoprofen (CAS# 22071-15-4) ^a	
	Tenoxicam (CAS# 59804-37-4) ^b	
	Naproxen (CAS# 22204-53-1) ^a	
	Indometacin (CAS# 53-86-1) ^a	

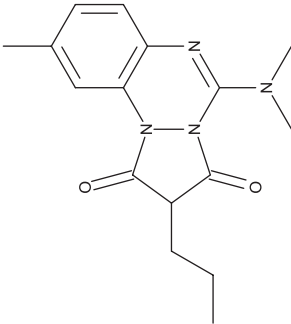
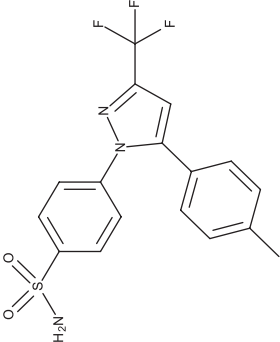
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Table 13.3 (Continued) Summary of Selected Bisphosphonates

Compound Class	Compound	Structure
Non-Selective NSAIDs (COX-1 and COX-2 inhibitors) (Continued)	Diflunisal (CAS# 22494-42-4) ^a	
	Piroxicam (CAS# 36322-90-4) ^a	
	Ketorolac (CAS# 74103-06-3) ^a	

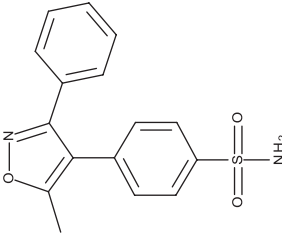
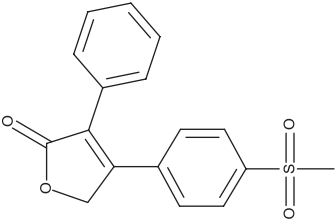
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Table 13.3 (Continued) Summary of Selected Bisphosphates

Compound Class	Compound	Structure
Non-Selective NSAIDs (COX-1 and COX-2 inhibitors) (Continued)	Azapropazone (CAS# 13539-59-8) ^c	
	Celecoxib (CAS# 169590-42-5) ^a	

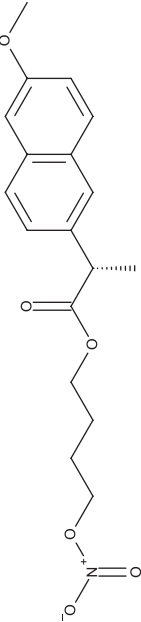
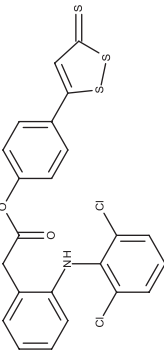
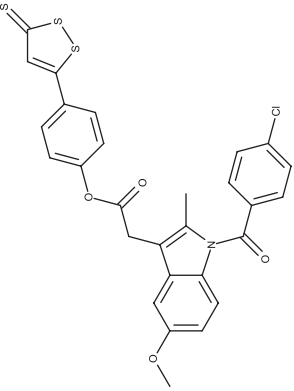
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Table 13.3 (Continued) Summary of Selected Bisphosphates

Compound Class	Compound	Structure
Selective NSAIDs (COX-2 inhibitors) (Continued)	Valdecoxib (CAS# 191695-72-7) ^d	
	Rofecoxib (CAS# 162011-90-7) ^d	

(Continued)

Table 13.3 (Continued) Summary of Selected Bisphosphonates

Compound Class	Compound	Structure
Nitric Oxide Releasing NSAIDs	Naproxcinod (CAS# 163133-43-5) ^e	
Hydrogen Sulfide Releasing NSAIDs	H2S-releasing Diclofenac ^d	
	H2S-releasing Indomethacin ^f	

^a Not approved by U.S. FDA; only available in EU as a second-line treatment for a maximum of 15 days, 100 mg bid/day.
^b Not Approved by U.S. FDA; Approved for use in EU for a maximum of 14 days, 20 mg/day.
^c Withdrawn from the market.
^d Voluntarily removed from U.S. market upon request of U.S. FDA.
^e Marketing authorization application withdrawn in EMA.
^f Untested in clinical trials.

infection (Bruno et al., 2014; Wolfe et al., 1999). Toxicities may occur in both the upper and lower GI tract and are discussed further below.

Upper GI Tract Toxicities

Upper GI toxicities include dyspepsia, damage to the protective gastric and duodenal mucosa, asymptomatic mucosal lesions, gastric and gastroduodenal ulcers, GI bleeding, and in some cases, death. Approximately 30%–50% of NSAID users develop asymptomatic subepithelial hemorrhages, erosions, and ulcerations primarily in the gastric antrum that reduce with chronic use or cessation of therapy and are not expected to elicit serious clinical toxicities. However, about 40% of NSAID users experience symptoms of gastroesophageal reflux disease and dyspepsia (belching, discomfort, bloating, and nausea) and approximately 10%–25% of chronic users develop gastric and duodenal ulcers (Sostres et al., 2013; Wolfe et al., 1999). Several meta-analyses have generally indicated that COX-2 specific NSAIDs elicit fewer upper and lower GI toxicities; however, these compounds have also been associated with increased risk of cardiovascular toxicities, requiring removal from the U.S. market in some cases (see [Table 13.3](#)). Toxicity associated with traditional NSAIDs varies based upon formulation, plasma half-life, and dose required for efficacy (Gargallo & Lanas, 2013). Massó González et al. assessed the risk of upper GI bleeding and perforation associated with traditional NSAIDs and COX-2 specific NSAIDs, determining overall RRs of 4.50 (95% CI, 3.82–5.31) and 1.88 (95% CI 0.96–3.71) for upper GI bleeding/perforation associated with use of traditional NSAIDs and COX-2 specific NSAIDs, respectively. Compound-specific RRs indicated that ibuprofen (2.69, 95% CI 2.17–3.33), rofecoxib (RR: 2.12 95% CI 1.59–2.84), aceclofenac (RR: 1.44 95% CI 0.65–3.2), and celecoxib (RR: 1.42 95% CI 0.85–2.37) exhibited the lowest RRs, while ketorolac (14.54 95% CI 5.87–36.04) and piroxicam (RR: 9.94 95% CI 5.99–16.50) exhibited the highest RRs, suggesting that COX-2 specific NSAIDs and ibuprofen are potentially less toxic to the GI tract. Massó González et al. also found that among traditional NSAIDs, the RR associated with low or mid doses (2.79 95% CI 2.17–3.58) was lower than the RR for patients receiving high doses (5.36 95% CI 4.57–6.29), indicating that dose level may play a significant role in observed toxicities. Furthermore, RRs associated with traditional NSAIDs with plasma half-life values exceeding 12 hours or slow release formulations were higher than NSAIDs with plasma half-life values less than 12 hours, demonstrating the potential impacts of drug formulation and physicochemical properties on GI toxicity (Masso Gonzalez et al., 2010). Castellsague et al. determined RR values associated with general risk of upper GI complications, including gastric ulcer perforations, obstructions, and bleeding for 16 traditional and COX-2 specific NSAIDs. Similar to results obtained by Massó González et al., high-dose NSAID therapy was generally associated with a 2–3-fold increased risk of upper GI complications compared to low or mid dose therapy, a relationship also observed for individual drugs. RRs ranged from 1.43 95% CI 0.65–3.15 for aceclofenac to 18.45 95% CI 10.99–30.97 for azapropazone. RRs for other NSAIDs stratified the compounds into groups with RRs <2, ranging 2–4, ranging 4–5, and exceeding 5. Compounds associated with the fewest upper GI complications included aceclofenac, celecoxib,

and ibuprofen, while piroxicam, ketorolac, and azapropazone were associated with the greatest risk. These meta-analyses exhibited general concordance in stratifying NSAIDs by GI safety profile, providing valuable information for researchers and clinicians aiming to limit or prevent NSAID-induced GI toxicity (Castellsague et al., 2012).

In addition to carefully considering which NSAID to administer to patients based upon safety profile, co-administration of gastroprotective agents such as misoprostol, proton pump inhibitors (PPI), and histamine-2 receptor antagonists with traditional NSAIDs have been associated with reduced upper GI toxicities, although strong clinical evidence has not been fully elucidated for use of these agents. Yuan et al. performed a systematic review of 82 clinical trials comparing use of traditional NSAIDs, COX-2 specific NSAIDs, and either class of NSAID concomitant with gastroprotective agents (proton pump inhibitors, histamine-2-receptor antagonists, and misoprostol) and found that combination therapy with COX-2-specific NSAIDs plus a PPI significantly lowers risk of GI ulcer complications with a risk ratio (RR) of 0.07, 95% confidence interval (95% CI) 0.02–0.18. Compared to treatment with traditional NSAIDs alone, COX-2 specific NSAIDs (RR: 0.25, 95% CI 0.15–0.38), traditional NSAIDs plus a PPI (RR: 0.28, 95% CI 0.12–0.41), traditional NSAIDs plus misoprostol (RR: 0.47, 95% CI 0.24–0.81), and traditional NSAIDs plus histamine-2-receptor antagonists (RR: 0.84, 95% CI 0.37–1.69) exhibited varying efficacy in preventing ulcer complications; NSAIDs plus misoprostol or histamine-2-receptor antagonists did not exhibit strong efficacy, suggesting that these therapies may not be protective against ulcer formation and complications (Yuan et al., 2016).

Lower GI Tract Toxicities

Lower GI tract toxicities primarily affecting the small intestine and colon account for nearly 40% of all serious GI events in patients administered NSAIDs. Capsule endoscopy (CE) studies, which provide photos of the duodenum, jejunum, and ileum, have identified serious intestinal injury and other lower GI adverse effects in patients administered NSAIDs over short and chronic periods (Lim & Yang, 2012). Goldstein et al. assessed the number of small bowel mucosal breaks induced by 200 mg/bid celecoxib (COX-2 selective NSAID) or 500 mg/bid naproxen with 20 mg omeprazole (proton-pump inhibitor) in GI lesion-free healthy volunteers. Results indicated that small bowel mucosal breaks associated with celecoxib treatment (16% of subjects, 0.32 ± 0.10 lesions/subject) were significantly lower compared to placebo or naproxen groups, in which 7% (0.11 ± 0.04 lesions/subject) and 55% (2.99 ± 0.51 lesions/subject) of subjects, respectively, developed small bowel lesions over a 2-week period, suggesting that (1) COX-2 specific inhibitors may be comparatively less GI toxic and (2) concomitant use of a proton-pump inhibitor was non-protective for NSAID-induced lower GI injuries (Goldstein et al., 2005). Maiden et al., (2005) observed macroscopic injuries to the small intestine, including mucosal breaks, redness, petechiae, denuded mucosa, and intestinal bleeding, in 68% of healthy subjects administered 75 mg slow-releasing diclofenac/bid and 20 mg omeprazole/bid over two weeks. A study in chronic NSAID users (>3 months use) with arthritis determined that 71% of patients exhibited small

bowel damage, which ranged from presence of red spots to large erosions and ulceration in patients (Graham et al., 2005).

OPIATES

Clinical Use of Opiates

Opiate drugs are widely used for management of pain resulting from cancer, post-operative pain, and chronic non-cancer pain, despite known potential for habituation and addiction. A variety of therapeutic and adverse effects associated with endogenous opioids and opiate drugs are primarily mediated through receptor binding to three major classes of G-protein coupled receptors including the δ -opioid receptor (OP1), kappa opioid receptor (OP2), and μ -opioid receptor (OP3), which are localized to the CNS and peripheral tissues including the gastrointestinal tract. OP1 is associated with mediating spinal and supraspinal analgesia, in addition to dopamine release required for amphetamine associated motor activity. OP2 is active within the spinal cord and brain, and results in pupil constriction, spinal analgesia, and inhibition of anti-diuretic hormone when activated. OP3 activity plays a role in supraspinal analgesia, respiratory depression, psychoactive effects, and a number of gastrointestinal effects. In humans, the most prevalent receptor in the gastrointestinal tract is the OP3 receptor, which may interact with endogenous opioid peptides such as met-enkephalin, leu-enkephalin, β -endorphin, and dynorphin as well as opiate agonists, partial agonists, and antagonist compounds. In the presence of opiate agonists in the GI tract, OP3 receptor-ligand complexes are endocytosed in a concentration-dependent manner and evoke constipation and slowed GI transit due to decreased peristalsis, which may be severe enough to warrant cessation of opiate use in some patients (Holzer, 2007; Pathan & Williams, 2012; Seifert, 2004; Smith, 2009).

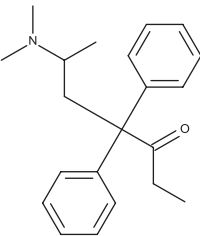
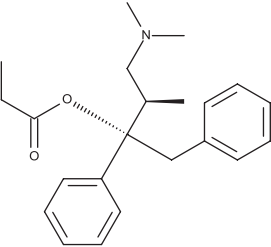
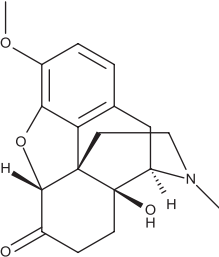
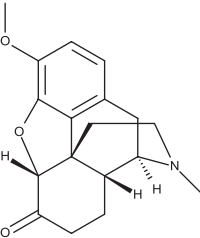
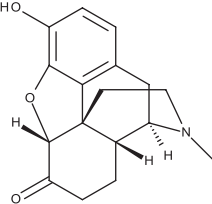
The opiate drug class is primarily divided into several major groups based upon structure and examples of common opiates are located in [Table 13.4](#).

Mechanisms of opiate metabolism may vary by both compound and individual variation observed in humans. Opiates are formulated in immediate release and sustained release forms, resulting in altered pharmacokinetic parameters. However, general patterns of metabolism have been elucidated for this class of drugs. A majority of orally administered opiates undergo extensive hepatic first-pass metabolism, significantly reducing bioavailability of these largely lipophilic compounds. Metabolism may occur via phase I or phase II reactions, primarily via CYP3A4 and CYP2D6 enzyme-mediated demethylation and UGT2B7-mediated glucuronidation, respectively (Holmquist, 2009; Smith, 2009). Terminal half-life values vary per compound, and are affected by route of administration, formulation, and co-administration of other drugs (especially those metabolized via similar pathways).

Mechanisms of Toxicity

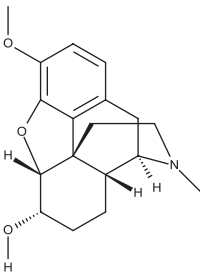
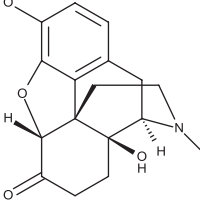
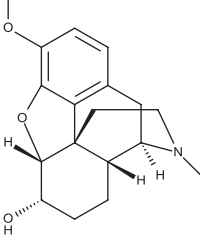
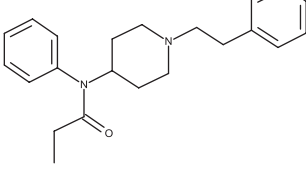
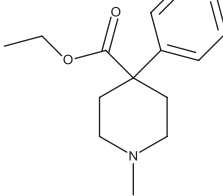
The gastrointestinal tract is a primary site of off-target action of opiate drugs due to prevalence of opioid receptors and endogenous opioids within the enteric nervous

Table 13.4 Summary of Selected Opiates

Compound Class	Compound	Structure
Phenylheptylamines	Methadone (CAS# 76-99-3)	
	Propoxyphene (CAS# 469-62-5) ^a	
Phenanthrenes	Oxycodone (CAS# 76-42-6)	
	Hydrocodone (CAS# 125-29-1)	
	Hydromorphone (CAS# 466-99-9)	

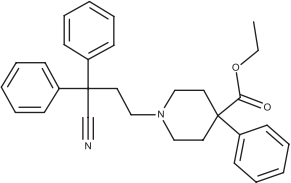
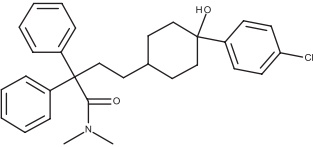
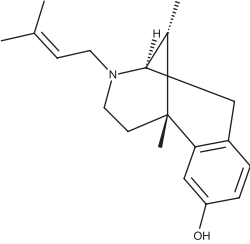
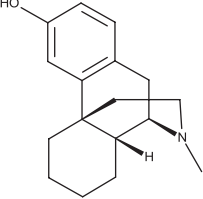
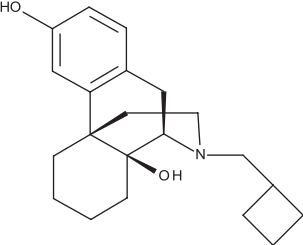
(Continued)

Table 13.4 (Continued) Summary of Selected Opiates

Compound Class	Compound	Structure
Phenanthrenes (Continued)	Morphine (CAS# 57-27-2)	 <chem>CN1CC[C@]23c4c5ccc(OC)c6ccccc4O[C@H]2[C@H]3CC[C@@H]1[C@H]5CC[C@H]6</chem>
	Oxymorphone (CAS# 76-41-5)	 <chem>CN1CC[C@]23c4c5ccc(O)c6ccccc4O[C@H]2[C@H]3CC[C@@H]1[C@H]5CC(=O)C6</chem>
	Codeine (CAS# 76-57-3)	 <chem>CN1CC[C@]23c4c5ccc(OC)c6ccccc4O[C@H]2[C@H]3CC[C@@H]1[C@H]5CC[C@H]6</chem>
	Fentanyl (CAS# 437-38-7)	 <chem>CC(=O)N(c1ccccc1)C2CCN(CC2)CCc3ccccc3</chem>
Phenylpiperidines	Meperidine (CAS# 57-42-1)	 <chem>CCOC(=O)C1(c2ccccc2)CCN(C)CC1</chem>

(Continued)

Table 13.4 (Continued) Summary of Selected Opiates

Compound Class	Compound	Structure
Phenylpiperidines (Continued)	Diphenoxylate (CAS# 915-30-0)	
	Loperamide (CAS# 53179-11-6)	
Benzomorphans	Pentazocine (CAS# 359-83-1)	
Morphinans	Levorphanol (CAS# 77-07-6)	
	Butorphanol (CAS# 42408-82-2) ^a	

^a Withdrawn from market by U.S. FDA.

system, which regulates gastrointestinal motility and secretion. Opioid receptors, especially the μ -opioid receptor, are responsive to endogenous and exogenous opioids and opiate drugs, therefore suggesting that peripheral μ -receptor antagonists would be of great therapeutic value to reduce adverse effects while maintaining desired CNS analgesia. Endogenous and exogenous opioids (i.e., μ -opioid antagonists, more specifically) disrupt propulsive peristalsis which decreases movement of chyme through the intestines, resulting in increased absorption of liquids and subsequently hardened stools in addition to reduced mucosal secretion, leading to the observed opiate-induced effects on the GI tract (Holzer, 2007).

Two principal gastrointestinal syndromes are observed in patients administered opiates: opiate-induced bowel dysfunction (OBD) and postoperative ileus. Additional gastrointestinal syndromes, including opiate-induced constipation (OIC) and narcotic bowel syndrome (NBS) have also been reported in patients administered opiates.

OBD is characterized by a full suite of μ -opiate receptor-mediated effects such as anorexia, nausea, vomiting, decreased gastric motility, altered mucosal secretion, gastroesophageal reflux disease (GERD), OIC, ileus, pseudo-obstruction of the bowel, bloating, and straining that persist throughout opiate treatment without development of tolerance (Holzer, 2009; Kurlander & Drossman, 2014; Kurz & Sessler, 2003). In a meta-analysis of 11 randomized trials, nearly 41% of patients taking an oral opiate up to 8 weeks developed symptoms of OIC or OBD. More specifically, constipation has been widely observed in clinical trials, affecting up to 70% of patients in some studies, and typically increases in prevalence with increased duration of therapy (Camilleri, 2011; Moore & McQuay, 2005). Furthermore, constipation may occur after a single opiate dose and is induced at much lower doses than those required for analgesia, indicating that dose reduction will not alleviate this adverse effect (Mehendale & Yuan, 2006). Postoperative ileus, which manifests as temporary (48–72 h) motor stasis of the intestinal tract, occurs universally after abdominal surgery, especially following procedures that require manipulation of the abdominal organs. This condition, which is worsened by concomitant use of opiates for pain management following surgery, results in bowel distension, gas, nausea, vomiting, severe constipation, abdominal cramping, and loss of appetite. As opiates have profound effects on gastrointestinal motility, use of these compounds for post-operative analgesia further complicates return to normal gastrointestinal function and often leads to increased recovery time and duration of stay in hospitals (Delaney, 2004; Holzer, 2007).

OBD/OIC and postoperative ileus are commonly treated to limited effect with standard laxatives or prokinetic drugs. A survey of patients taking opiates for non-cancer pain found that only 46% of subjects requiring laxatives for OIC reported adequate relief >50% of the time, suggesting that alternative therapies are required for treatment of this adverse effect (Camilleri, 2011; Pappagallo, 2001). The value of OBD and postoperative ileus treatment with prokinetic drugs, which may stimulate GI motility, intestinal tone, or colonic transit, has not been fully substantiated clinically (Holzer, 2007). Therefore, management of OBD and postoperative ileus has shifted towards use of peripherally acting μ -opioid receptor antagonists (PAMORAs), which cannot cross the blood-brain barrier and permits desired analgesic activity of

opiates in the CNS. PAMORAs, including naloxegol (Movantik®, formerly NKTR-118), methylnaltrexone (Relistor®), and naldemedine (Symproic®), as well as alvimopan (Entereg®) have been FDA-approved for use in treating opiate-induced constipation and post-operative ileus, respectively (Camilleri, 2011; Holzer, 2007, 2009). Lubiprostone (Amitiza®), a chloride channel activator that induces intestinal secretions, promotes increased passage of stool aiding in treatment of opiate-induced GI effects (Camilleri, 2011).

Although not as prevalent as OBD or postoperative ileus, approximately 4.2%–6.4% of chronic opiate patients develop NBS, which is characterized by incompletely or poorly controlled abdominal pain with increased doses of opiates. Symptoms of NBS are not mediated peripherally by μ -opioid receptors; instead, effects may be related to opiate-induced hyperalgesia (OIH) induced by the CNS effects of opiates on nociception. Chronic opiate use may modulate neuronal plasticity, altering the function of central neuronal systems responsible for analgesia towards enhanced nociception or hyperalgesia. Effective treatments do not exist for NBD, aside from opiate detoxification that may be undesirable if pain management is still required for other conditions (Kurlander & Drossman, 2014).

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CHAPTER 14

Species Differences in GI Toxicity

Shayne C. Gad

CONTENTS

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The use of animal models in the nonclinical assessment of safety of agents potentially effecting the gastrointestinal (GI) tract (and using it as a portal to the body) can be classified as being for one of two objectives (Gad, 2007; Hall and Shapiro, 2010; Gad, 2015; Jiminez et al., 2015).

First, as part of a general program for assessing the systemic toxicity of a new drug, food, or other material for potential human use. In this case, such studies are almost always not focused specifically on detecting adverse effects on the GI tract—rather, they are effectively broad screens for potential toxicity. In such cases, model selection is most likely either by custom or based on the animal models being pharmacologically responsive to the agent being tested. In this case, a concern is that one needs to understand how effects seen in the GI tract of a model species are relevant (or not) to expectations of what should be expected in humans (or other target species of concern). In such cases, in the absence of knowledge of any known species-specific advantages (such as prior use for other member of the same chemical class, presence of relevant pharmacologic receptors in the animal species, or intolerance to the therapeutic molecule or a component of the formulation), we usually default to the most commonly used species. When it comes to understanding the results of such studies, we usually refer to [Tables 14.1](#) and [14.2](#)

Table 14.1 Comparison of Lengths of the Intestinal Tract and Its Major Subdivisions in Seven Different Species

Region of Intestinal Tract	Mouse			Rat			Rabbit			Dog			Pig			Human		
	Length (cm)	% of Total	Length (cm)	Length (cm)	% of Total	% of Subd	Length (cm)	% of Total	% of Subd	Length (cm)	% of Total	% of Subd	Length (cm)	% of Total	% of Subd	Length (cm)	% of Total	% of Subd
Duodenum	5.3 ^a		9.5–10 ^b	—		8	—		—	25 ^{c,d}		6	—			25 ^e		4
Jejunum	46.2 ^a		90–135 ^b	—		90	—		—	360		90	—			260 ^a		38
Ileum	—		2.5–3.5 ^b	—		2	—		—	15 ^{c,d}		4	—			395 ^a		58
Total small intestine	45	~75	125 ^b	83	339–398 ^{f,c}	61	—		—	~400 ^{c,g,h}	83	—	1,500–2,000 ^{c,g,h}	70–85	81	680 ^a		
Cecum	3.4 ^a		5–7 ^b	26	41–61 ^{c,g,i}	28	—		—	5–8 ^{c,d,g}	~11	—	20–30 ^{c,g,h}	7 ^e		7 ^e		5
Colon	10.0 ^a		9–11 ^b	42	114–165 ^{c,g,i,j}	72	—		—	25–60 ^{c,d,g,h}	~81	—	400–500 ^{c,g,h}	93 ^a		93 ^a		60
Rectum	—		8 ^b	32	—	—	—		—	5 ^d	~8	—	—	55 ^e		55 ^e		35
Total large intestine	—	~25	25 ^b	17	—	39	—		—	~82	17	—	350–850	15–30	19	155 ^e		
Total intestinal tract	—		150 ^b	582 ^{c,g}			—		—	482 ^{c,g}			2,350 ^{c,g}			835 ^a		

Source: Adopted from DeSesso, J.M. and Williams, A.L., Contrasting the gastrointestinal tracts of mammals: Factors that influence absorption, in Macor, J.E. (Ed.) *Annual Reports in Medicinal Chemistry*, Vol 43, Elsevier, Philadelphia, PA, pp. 353–371, 2008.

^a Ogiolda et al. (1998); intestinal segments unfixed.
^b Hebel and Stromberg (1986).
^c Karali (1995).
^d Evans (1993).
^e Snyder et al. (1975).
^f Pappenheimer (1998).
^g Stevens (1977).
^h Dressman and Yamada (1991).
ⁱ Snipes (1997); formaldehyde fixed segments.
^j Snipes et al. (1982); Bouins solution fixed segments.

Table 14.2 **Calculated Total Small Intestinal Surface Areas for Seven Species**

	Bodyweights (kg)	Smooth Luminal Surface Area (m ²)	Fold-Increase Factors			Estimated Total Surface Area (m ²)
			Plicae	Villi	Micro-villi	
Mouse	0.032	0.004 ^a	(1) ^b	(5) ^b	(20) ^b	(0.4)
Rat	0.3	0.016 ^a	1 ^c	5 ^c	20 ^c	1.6
Rabbit	2.6–3.9	0.087–0.096 ^a	1 ^d	5.7 ^e	24 ^f	11.9–13.1
Dog	10.7–12.6	0.099–0.14 ^{a,g}	1 ^d	10 ^h	25 ⁱ	24.75–35
Pig	47	1.4 ^g	1 ^d	6 ^j	(20–25) ^k	(168–210)
Monkey	2.7–3.0	0.091–0.11 ^{g,l}	(3) ^m	(10) ^m	(20) ^m	(54.6–66)
Human	70	0.42 ^a	3 ^c	10 ^c	20 ^c	252

^a Values as reported in Pappenheimer (1998).
^b Extrapolated from values for rat.
^c From DeSesso and Jacobson (2001).
^d Value based on the absence of plicae in the small intestine.
^e Value based on comparison of callus lengths between rabbits and humans as reported in Thomson (1986), and DeSesso and Jacobson (2001).
^f As cited in Westergaard and Dietschy (1974).
^g Values as reported in Chivers and Hladik (1980).
^h Based on same size villi as humans as reported in DeSesso and Jacobson (2001), and Paulson et al. (2003).
ⁱ As reported in Kararli (1995).
^j Based on comparison of villi lengths between pigs and humans as reported in DeSesso and Jacobson (2001), and Shirkey et al. (2006).
^k Extrapolated from values reported for other animal species.
^l Values as reported for cynomolgus monkeys.
^m Extrapolated from values for human.

for general species GI characteristics. We might, however, also consider factors dictated by experience (see also [Table 14.3](#)):

1. Dogs are very prone to emesis (Dressman et al., 1993; Evans, 1993).
2. Dogs, pigs, and primates (like humans) have a single-chamber stomach. This is not the case with the other common model species.
3. On the other hand, rats and mice lack the musculature to be able to demonstrate emesis. The best model for evaluating potential emesis in humans is the ferret.
4. Rabbits are herbivores, and have much longer cecum and the longest of the model species.
5. All the common laboratory species are more sensitive to the GI irritation (and more extreme) effects of nonsteroidal drugs and cox-inhibitors.

The second case is if there is a specific reason to expect, or need to evaluate or understand, the GI tract as a potential target organ for an agent. This could be from a safety pharmacology aspect, because of an observation in earlier studies, or because the agent being evaluated is intended to have effects on the GI tract, or, particularly with the increasing recognition of acute and chronic inflammation as necessary (and even sufficient) precursors to serous systemic diseases, the GI tract presents a specific area of investigative focus (Jiminez et al., 2015).

The inflammatory response is primarily an expression of the innate immune system, but this response also is an expression of the coordination between

Table 14.3 Advantages and Disadvantages of Various Animal Models Used to Study Intestinal Inflammation Based on the Immune Response to Toxicants and Biological Challenges

Animal Model	Type	Immune Mechanism	Incitant	Advantage	Disadvantage
Zebratfish	Invertebrate	AI	C	• Cost-effective • Adaptive immunity a factor at 3 weeks of age • Minimal ethical considerations • Similar microbial community • Germ-free, transparent embryo/larvae • Simple husbandry • Easy genetic manipulation • Microbiome studies possible • Germ-free • Gut-brain axis studies • Use of adequate numbers for statistical relevance • Easy genetic manipulation • Microbiome studies possible • Intestinal loop models applicable • Easy genetic manipulation • Microbiome studies possible • Anatomically similar to humans • Germ-free • Gut-brain-axis studies	• Small tissue/size • Different nutritional requirements • Cost of germ-free facilities • Cannot do diet or nutritional comparisons
Rodent	Vertebrate	AI	BC	• Simple husbandry • Easy genetic manipulation • Microbiome studies possible • Germ-free • Gut-brain axis studies • Use of adequate numbers for statistical relevance • Easy genetic manipulation • Microbiome studies possible • Intestinal loop models applicable • Easy genetic manipulation • Microbiome studies possible • Anatomically similar to humans • Germ-free • Gut-brain-axis studies	• Small tissue size in murine model • Coprophagy limitation • Difficult for invasive surgical techniques • Cost and establishment of Level 2/germ-free handling facilities • Cost and establishment of Level 2 handling facilities • Longer gestation periods • Cost and establishment of Level 2/germ-free handling facilities • Overwhelming amount of tissue • Husbandry and personnel training • Slight variation in immune cells and large intestine orientation • Level 2 handling facilities • Overwhelming amount of tissue • Husbandry and personnel training • Intestine not well studied • Microbial fermentation occurs in the rumen rather than the cecum/colon • Ethical considerations • Level 2 handling facilities • Disease transmission • Husbandry and personnel training • Overwhelming amount of tissue
Dog/Guinea Pig/Rabbit	Vertebrate	AI	BC	• Easy genetic manipulation • Microbiome studies possible • Intestinal loop models applicable • Easy genetic manipulation • Microbiome studies possible • Anatomically similar to humans • Germ-free • Gut-brain-axis studies	
Swine	Vertebrate	AI	BC	• Easy genetic manipulation • Microbiome studies possible • Anatomically similar to humans • Germ-free • Gut-brain-axis studies	
Ruminant	Vertebrate	AI	BC	• Intestinal loop models available • Beneficial for enteric pathogen studies i.e. O157:H7	
Non-human primate	Vertebrate	AI	BC	• Anatomically and genetically similar to humans • Gut-brain axis studies • Microbiome studies possible • Human disease present with similar sequelae to human	

A, adaptive immune response with associated mechanism; I, innate response with associated immune mechanism; B, biological incitant; C, chemical incitant.

the complex innate and adaptive immune systems associated with the GI tract (Geremia et al., 2014; Wallace et al., 2014).

A starting place for model selection should always be considered as one of the primary functions of the GI tract.

- A. As a route of entry for nutrients (and desirable other exogenous compounds such as drugs) into the body
- B. As a barrier to entry of organisms and undesirable molecules
- C. As a specific portion of the immune system acting to support function “B”
- D. As the home of the microbiome community in the body, allowing those microorganisms to function and thrive in support of digestion and metabolism of desirable nutrients and assist in the tracts barrier to threat function

As presented elsewhere in this book, pigs (and, in particular, minipigs) are for most purposes a more relevant model of gastrointestinal toxicity than the other common model species. Key similarities in pig and human gastrointestinal physiology are listed below.

- Stomach and small intestine anatomically similar
- pH gradient is similar (see Table 14.4)
- Transit time (see Table 14.5)
- Key for orally administered drugs and studying drug delivery systems that rely on above factors
- Passive diffusion in the small intestine is similar to humans

Table 14.4 Range of pH Values Reported for Different Portions of the Gastrointestinal Tract for Seven Species of Animals^a

Species	Stomach		Small Intestine ^b			Large Intestine		
	Anterior	Posterior	Duodenum	Jejunum	Ileum	Cecum	Colon	Rectum/ Feces
Mouse	4.5	3.1	—	—	—	—	—	—
Rat	4.3–5.1	2.3–4.0	6.5–7.1	6.7–6.8	7.1–8.0	6.4–7.6	6.6–7.6	6.9
Dog	1.5–5.5	1.5–3.4	6.2	6.2–7.3	7.5	6.4	6.5	6.2
Rabbit	1.9–2.2	1.9–2.2	6.0–6.1	6.8–7.5	7.2–8.0	5.7–6.6	6.1–7.2	7.2
Pig	1.6–4.3	6.0	6.2–6.9	7.5	6.3	6.8	7.1	—
Monkey	4.7–5.0	2.3–2.8	5.6–6.0	5.8–6.0	6.0–6.7	4.9–5.1	5.0–5.9	5.5
Human	1.5–5.0	5.0–7.0	6.0–7.0	7.0–7.4	5.7–5.9	5.5–7.5	6.5–7.0	—

^a The range of values reported here are from the following references: Calabrese (1983); Lui et al. (1986); Fallingborg et al. (1989); Fekete (1989); Bruorton et al. (1991); Kararli (1995).

^b Some studies reported pH values for segments 1,3,5, and 7 of the small intestine. For the purposes of this table, segment 1 was designated the duodenum, segments 3 and 5 were designated the jejunum and segment 7 was designated the ileum.

Table 14.5 Range of Gastrointestinal Transit Times (hrs) through Different Portions of the Gastrointestinal Tract as Reported for Seven Species

Species	Oro-Cecal						
	Stomach		Small Intestine		Large Intestine		Total
	Liquid	Solid	Liquid	Solid	Liquid	Solid	
Mouse	—	—	—	—	—	—	—
Rat	0.7–2.1 ^a		2.6–3.3 ^a		15.5 ^b		38.9 ^c
Dog	1.7 ^d		—	—	18.5 ^e		270–374 ^{e,f,g}
		14.3 ^e					
Rabbit		9.1 ^h			9.6 ^h		18.1–20.6 ^h
Pig	0.8–0.9 ⁱ	1.0–1.3 ⁱ	3.9–4.4 ⁱ	3.7–4.3 ⁱ	24.9–41.3 ^j	35.6–44.4 ⁱ	29.1–46.6 ^j
Monkey		1.8 ^j /2.3–2.5 ^k			—	—	40.5–49.7 ⁱ

(Continued)

Table 14.5 (Continued) Range of Gastrointestinal Transit Times (hrs) through Different Portions of the Gastrointestinal Tract as Reported for Seven Species

Species	Oro-Cecal					
	Stomach		Small Intestine		Large Intestine	
	Liquid	Solid	Liquid	Solid	Liquid	Solid
Human	Child	1.1 ^l	7.5 ^q		172–40 ^{l,m}	—
	Young adult	1.0–1.6 ^{d,n,o,p}	1.9–2.3 ⁿ	3.4–3.5 ⁿ	35.0 ⁿ	—
	Mature adult	0.8–1.1 ⁿ	1.3–1.8 ⁿ	2.0–4.2 ⁿ	33.5–61.5 ^{n,r}	—

^a Measurements from fed rats (Tuleu et al., 1999).
^b Measured by the insertion of dye in the cecum (Enck et al., 1989).
^c Sastry and Rao (1991).
^d Liquid transit in fasted Beagle dogs and young adults (21–29 years of age) (Lui et al., 1986).
^e Measured in standard Schnauzers (Hernot et al., 2006).
^f Measured in Beagle dogs (Burrows et al., 1982).
^g Measured in French Bulldogs, English Cocker Spaniels, and standard Schnauzers (Hernot et al., 2005).
^h Gidenne and Ruckebusch (1989).
ⁱ Measurements derived from pigs with average weight of 33 kg (Wilfart et al., 2007).
^j Measurements from fed cynomolgus monkeys (Kondo et al., 2003a).
^k Measurements from fasted cynomolgus monkeys (Kondo et al., 2003b).
^l Determined in children 8–14 years of age (Fallingborg et al., 1990).
^m Determined in children 4–15 years of age (Wagener et al., 2004).
ⁿ Determined in adults 20–30 years of age (young) and 38–53 years of age (mature) (Graff et al., 2001).
^o Ewe et al. (1989).
^p Fallingborg et al. (1989).
^q Measurements for males and females, 19–45 years of age (Chiou et al., 2000).
^r Males only (Cummins et al., 1978).

Other considerations:

- Less prone to vomiting (compared to dogs)
- Larger blood volume for repeated PK sampling
- Cost/availability (particularly accepted in EU)

It should be noted, however, that there are significant differences between the different species of minipig, and that minipigs are easy to stress, rapidly leading to prompt changes in GI function.

Oral studies in primates are uncommon because of both difficulties in conduct and limited relevance of results to human effects (Valentin et al., 2015). There is also the growing societal reluctance to use primates in research.

A new consideration is the difference in GI microbiomes of the potential model species, and how the relevance of these applies to the human species.

If we consider relational biome populations and the nature and rate of effects of exogenous agent effects, it is only now that we have come to realize the importance of these bacterial flora as a component of the response of the GI tract and metabolic processing of these molecules (Bruorton et al., 1991; Bleich and Fox, 2015). From 500 to 1000 bacterial species may inhabit the intestines of a laboratory animal. The resulting combined biome can significantly affect the susceptibility to inflammatory responses and progression of toxin-induced effects directly on the GI tract, as well as comparative absorption of toxins into the body.

Chapter 13 on classes of chemicals known to have specific GI toxicities should also provide guidance as to which laboratory animal species have served to date as the best predictors of human health effects.

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Nanoparticles in the Gastrointestinal Tract

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The highly absorptive gastrointestinal tract (GIT) of the human and other animal species is very well suited for the absorption of small particles with dimensions of less than 1 μm [1,2]. Humans along with most all living things evolved in an environment that included daily oral exposure to naturally occurring microparticles and nanoparticles to include dietary (lipid micelles, minerals, peptide and protein fragments, and other food particles of many types) and non-dietary substances. With the advancement of materials science and technology to include the ever-increasing development and utilization of engineered nanotechnology products (including food and therapeutics), GIT exposure to an ever-wider variety of nanomaterials (including man-made types) already is increasing both qualitatively and quantitatively. Moreover, unlike the situation with respirable nanoparticles that has been the subject of many toxicological investigations over the past two decades, relatively little is known about the gastrointestinal intake and handling of most nanoparticles, especially those that are man-made or “engineered.” With increasing interest as to the effects of local and systemic exposure to nanomaterials from various possible routes, many toxicologists are now beginning to apply their expertise gained from the study of nanomaterials

exposures via other routes to the study of GIT nanoparticle exposures and the literature on exposure to nanomaterials via this route is growing. These scientific studies are addressing issues such as (1) the characterization of particle–host or particle–cell interactions, (2) the mechanisms of uptake of particles in the gastrointestinal tract, especially via the Peyer’s patches, (3) the cellular and systemic effects of nanoparticles to include effects on both micro- and macro-cellular and tissue biology and on biochemical process, (4) the generation of reactive oxygen species, (5) the activation of pro-inflammatory signaling, and (6) immune responsiveness mechanisms.

The “nanoscale” is generally accepted as including materials having at least one dimension between 1 and 100 nm. This is the size region where the quantal characteristics of the very small dimensions are most evident. There is some interest in expanding this range to include all materials having at least one dimension in the nanometer range of ≥ 1 nm to ≤ 999 nm; however, the essential and most practical biological consideration is what dimensionality for a given material does it exhibit the quantal characteristics of substances at the nanoscale.

Developing a common definition for nanomaterials has been challenging because of the need to satisfy two diverging considerations: (1) the definition should be broad enough to define materials that may warrant additional evaluation; (2) it should not be so broad as to include those materials for which additional examination or evaluation would not be meaningful in terms of protecting human health or the environment. Definitions proposed to date have taken a variety of approaches as they seek to support various and often specific jurisdictional mandates, and this frequently leads to proposals to incorporate contradictory inclusions and exclusions with the definition. This creates a complex regulatory maze for producers of nanomaterials and products containing them as to when to report whether or not a product contains nanomaterials. Therefore, a balance is necessary to ensure appropriate and practical coverage of emerging nanomaterials to most effectively protect the health and safety of humans and the environment while at the same time allowing for the potential useful applications of proven safe nanomaterials. Overcoming difficulties associated with attempting to comply with contradictory nanomaterial definitions and the different regulations where they are used can impede the development and entry of useful and safe nanomaterials into international trade, which may fundamentally reduce public confidence in the adequacy of regulatory protections from exposure to nanomaterials. This is exactly why many regulatory bodies have taken the position that nanomaterials be considered for safety on a case-by-case basis and broad classification schemes fail for materials in such a technologically expansive landscape.

The International Organization for Standardization (ISO) has descriptively defined “nanomaterial” as a “material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale” [3] and “nanoparticle” as a “nano-object having all three external dimensions in the nanoscale” where nanoscale is defined as the size range from approximately 1 to 100 nm [4]. These technical definitions, based on size only, may be insufficient from a risk evaluation standpoint because they do not include other important elements that should be considered when determining whether a nanomaterial may need additional review and scrutiny.

Nanomaterials are neither inherently hazardous nor inherently safe [5,6]. This has been broadly recognized and it is prudent that they should not be treated as such in evaluation programs [7–10].

In the broadest view and in modern toxicological terms, particles could be considered “nano” particles if the largest dimension of the particle was in the nano range of <999→1 nm [11]. This is a definition that has been used in some regulatory documents [12,13] to include essentially everything in the nanoscale. In fact, it is becoming increasingly evident from the growing body of scientific evidence that some particles above 100 nm still exhibit quantum behavior in addition to their chemical characteristics, such that absorption through biological membranes and diffusion in biological fluids that results in exposure is profoundly different than the same particle with even very low micrometer dimensions. This is often because, at least in part, some materials have a broad particulate range where the mean particle diameter is above 100 nm but the particle distribution includes a substantial proportion of particles <100 nm. This is illustrated below in more detail under the topic of nanomaterial characterization.

Nanoparticles are a subset of nanomaterials in general [14]. Figure 15.1 shows a practical scheme for the classification of different types of nanomaterials.

Figure 15.2 provides a biological perspective on nanomaterial dimensions in contrast to the dimensions of biological structures and biomolecules that is helpful in understanding how materials in this size range may affect normal biological processes [14].

The primary function of the GIT is generally recognized as the processing of food materials and absorption of essential nutrients to provide the materials required for cellular growth and metabolism and maintaining the homeostasis of normal systemic functions. The process of digestion and absorption in the GIT consists of a complex set of chemical interactions within the lumen of the tract as well as biologic

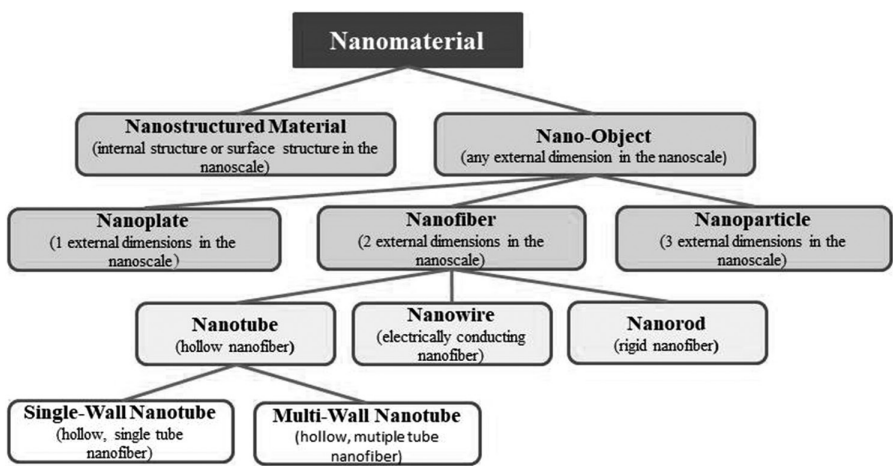


Figure 15.1 A practical scheme for the classification of different types of nanomaterials. (From Hobson, D.W. et al., *Int. J. Toxicol.*, 35, 5–16, 2016.)

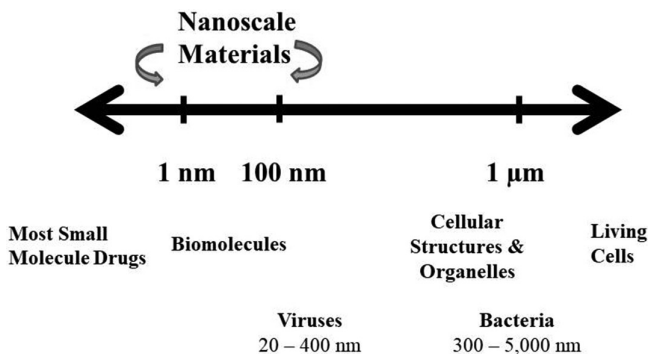


Figure 15.2 A biological perspective on nanomaterial dimensions in contrast to the dimensions of biological structures and biomolecules. (From Hobson, D.W. et al., *Int. J. Toxicol.*, 35, 5–16, 2016.)

interactions between the cells and tissues of the tract. These cellular mechanics are well suited to facilitate the transport of particulates, especially nanoparticles, from the GIT lumen into the body.

Since the physicochemical nature of nanoparticles with different sizes and compositions is affected by the environment in which a given particle is surrounded, it should not be difficult to understand that nanoparticles of different sizes and compositions would exhibit differential rates and degrees of absorption as they pass through the GI tract [15,16]. These characteristics may be helpful in determining whether or not a given nanomaterial a nutrient or a toxicant.

The degree to which a given nanoparticle might produce toxicity depends largely on how the digestive and absorptive functions of the GI system interact with the nanoparticle and the inherent toxicity of the particle, whether it be toxicant or toxin in nature. In this chapter, the absorption of essential nutrients will be described as well as the contrasting absorption and toxicological action of currently recognized nanoparticulate toxicants and toxins.

CHARACTERIZATION OF NANOMATERIALS

Nanotechnology is a rapidly emerging field of great interest and promise that is heavily engaged in the development of “engineered” nanomaterials for a very wide variety of applications. As new materials are developed and commercialized, hazard information also needs to be generated to reassure regulators, workers, and consumers that these materials can be used safely. The biological properties, including GIT absorption, of nanomaterials are closely tied to the physical characteristics, including size, shape, dissolution rate, agglomeration state, and surface chemistry, to name a few. Furthermore, these properties can be altered by the medium used

to suspend or disperse these water-insoluble particles. Unfortunately, some older toxicology literature often lacks much of the characterization information that toxicologists and regulators need to accurately assess potential hazards of emerging, engineered nanotechnologies. Toxicologists often need to know a relatively complex set of characteristics for a given particle to better investigate and understand its interactions with the biological system. A lack of adequate characterization also leads to different laboratories reporting discordant results on seemingly the same test nanomaterial because subtle differences in the particle characteristics lead to differences in the dispersion medium used that resulted in altered properties and variable toxicity of the particle. For these reasons, adequate characterization data should accompany and is now often required of all scientific publications concerned with nanomaterials biological effects.

The scientific community, regulatory agencies, environmentalists, and most industry representatives all agree that more effort is required to ensure the responsible and safe development of new nanotechnologies. Characterizing nanomaterials is a key aspect in this effort. There is no universally agreed-upon minimum set of characteristics although certain common properties are included in most recommendations. Therefore, characterization becomes more like a puzzle put together with various measurements rather than a single straightforward analytical measurement. In this chapter, we emphasize and illustrate the important elements of nanoparticle characterization with a systematic approach to physicochemical characterization—often with the inclusion of a comprehensive overview or summary describing the nanotechnological properties that are most significant to toxicological testing along with suggested methods for characterizing an as-received nanomaterial and then specifically addressing the measurement of size, surface properties, and imaging [17,18].

As indicated above, the need for proper characterization is essential to the advancement of reliable nanomaterial science including, toxicology, pharmacology, and food science to the extent that some scientific journals now have instructions to authors regarding adequate characterization of test materials, including nanomaterials, before an article will be accepted for publication [19,20]. Sufficient details of the nanomaterial characterization as well as the techniques employed to enable the work to be repeated with adequate fidelity to the original, reported, scientific study must be provided [19,20].

Some important practical considerations in evaluating the safety of nanomaterial use in new products including food and therapeutics are that

- The concern is generally for “unbound” nanomaterials.
- Many products may employ nanotechnology but may not require extensive safety evaluation if it can be established by design and relevant data that the nanomaterial is not bioavailable.
- *In vitro* diagnostic tests employing nanotechnology generally would not require *in vivo* safety or biocompatibility evaluation.
- The manufacturer is responsible for demonstrating clearly a lack of potential exposure.

Central to these practical considerations is the need to ensure adequate characterization of nanomaterials where exposure is possible to support state-of-the-art scientific and regulatory needs for good practices data. This characterization must include the evaluation of the physical parameters of nanomaterial dimensions, composition, surface characteristics, etc. and also both *in vitro* and *in vivo* interactions with biologic systems.

The physical characteristics that should be addressed for nanomaterials that may enter the GI tract as well as other routes of exposure include: size and size distribution, composition, structure and morphology, surface chemistry, macromolecular weight, surface area, porosity, solubility, surface charge density, purity, sterility, and stability.

In vitro characteristics that should be considered as warranted include the following:

- Binding
- Pharmacological effect in isolated cells and tissues
- Blood contact properties
- Cellular uptake, distribution, metabolism, and cytotoxicity
- Mutagenicity

In vivo assessment of nanomaterial characteristics would include:

- Absorption
- Pharmacokinetics
- Serum half-life
- Protein binding
- Tissue distribution
- Metabolism
- Elimination/Excretion
- Safety
- For route and indication
- Appropriate species
- Appropriate duration
- Relevant end points

The methodology used to address each of these characteristics includes a wide array of methods and protocols that are ever increasing and improving. The specific set of tests to be conducted is dependent on the actual nanomaterial with consideration to the intended use and conditions of exposure. Transmission electron microscopy, for example, is considered the standard for nanomaterial size characterization, but there are many other particle-size determination methods including dynamic light scattering (DLS) and hyperspectral imaging that rapidly provide both particle size and size distribution data with less time in sample preparation and analysis. The methods recommended to adequately support good scientific and regulatory practice are discussed in the growing current literature on this subject and are listed in regulatory guidance documents [17,18].

GIT ABSORPTION AND DISTRIBUTION OF NANOPARTICLES

The processes involved in the absorption of nanomaterials from the GIT are complex and may involve any of several different diffusion and transport mechanisms that differ somewhat in different regions of the GI tract. These mechanisms include simple diffusion, facilitated diffusion, active and passive transport, endocytosis, pinocytosis, and phagocytosis. Figure 15.3 contrasts the general locations where natural substances, including food and nutrients are absorbed in the GIT to locations where different, general types of nanomaterials have been reported to be absorbed.

The human GIT has an average length of approximately 5 meters from the buccal cavity to the rectum [21,22]. The buccal cavity, esophagus, stomach, small intestine, and large intestine are essentially macroscopic compartments that are separated by sphincters. The GIT primary functions in the maintenance of water homeostasis, the digestion, and absorption of macro- and micronutrients and electrolytes, the processing of the fraction of macromolecular antigens that survive digestion, and the exclusion of pathogens. Over most of its length, the GIT is lined with a multi-layer, stratified squamous epithelium that is well suited to handle the high volume

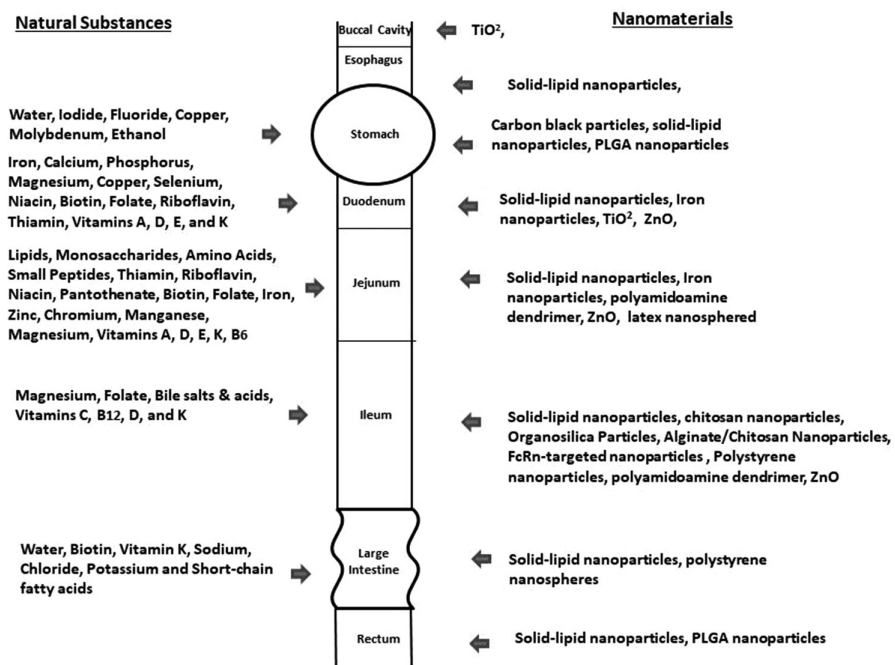


Figure 15.3 General locations where natural substances, including food and nutrients, are absorbed in the GIT in contrast to locations where different types of nanomaterials are reported to be absorbed.

of quickly passing food. The surface area of the GIT has typically been reported to be between 180 and 300 square meters; however, recent studies show that the surface area is significantly smaller and in healthy adults is “only” between 30 and 40 square meters [22].

Published information on the absorption rate of particulates through the epithelium of the buccal cavity and esophagus is sparse. This is understandable because the surface area is low, the residence time for most food matrices is short, and the intestine is more specialized for selective uptake of macromolecules in this size range. The morphology of buccal superficial cells determines the size-dependent uptake of nanoparticles in the oral cavity. The squamous epithelium exhibits furrows exhibit characteristic ridge-like folds of plasmalemma, termed microplicae, that have diameters in the range of 210–410 nm. Large nanoparticles/aggregates (showing comparable sizes) settle on the bottom of the furrows and are immediately internalized by the cell compared to smaller particles [23,24].

The surface area of the human stomach is approximately 0.05 square meters. Despite the relatively low surface area and permeability of the gastric epithelium to macromolecules, a minimal passage of nanoparticles is, nevertheless, allowed [21]. In the stomach and many other regions of the GIT, nanoparticles must first cross a mucus barrier before coming in contact with epithelial cells. Mucus is comprised of mucin glycoproteins that form viscoelastic gels resulting in an adherent unstirred layer coating the GI wall [23,25].

The mucus layer of the stomach and large intestine is bound tightly to the epithelial lining [24]. Adhesive substances are trapped in the mucus, but non-adherent materials can diffuse through this layer and make epithelial contact. In the small intestine the mucus layer is somewhat thinner than other GIT regions and there is less interaction between lamellar strings, allowing greater access of the luminal contents to the epithelium [21,26,27]. This permits the uptake of nutrients, while trapping, immobilizing, and excluding larger potentially hazardous particulates, e.g., bacteria, toxic, and irritant particles [21,28,29]. The small intestine includes three consecutive parts: the duodenum, jejunum, and ileum and is the longest segment of the GIT. The outermost microscopic layer of the small intestine is structured with villi and microvilli that project into the lumen, resulting in a large surface area of 30 square meters in humans [22,23].

The epithelial cell monolayer that covers the villi forms a tight but selective barrier to microbes and most macro-molecular antigens, but nutrients are easily absorbed with great efficiency. The epithelial monolayer contains several specialized cell types. Enterocyte cells are responsible for nutrient absorption, while other cell types perform functions such as the secretion of mucus [29].

The large intestine (colon) includes large (haustral) folds of the mucosa. They are formed by circumferential contraction of the inner muscular layer of the colon. The colon is much shorter and lacks the villi projections seen in the small intestine and has a surface area of 2 square meters with columnar enterocytes in the epithelium being the predominant cell type [21,22].

The gut-associated lymphoid tissue (GALT) is distributed in localized regions of the wall of the small and large intestine and is composed of isolated follicles and aggregated lymphoid follicles termed Peyer's patches [21]. In addition to enterocytes,

these tissues have a specialized epithelium containing antigen sampling (M)-cells that have a role in processing antigenic substances including nanoparticles and occur in a much thinner mucus layer allowing direct interfacing with lumen [21].

In general, nanoparticle absorption of food materials (as well as dietary nanomaterials) can be divided into three major phases:

1. The *luminal phase* is the phase in which dietary fats, proteins, and carbohydrates are hydrolyzed and solubilized by secreted digestive enzymes and bile.
2. The *mucosal phase* relies on the integrity of the brush-border membrane of intestinal epithelial cells to transport digested products from the lumen into the cells.
3. The *postabsorptive phase* in which reassembled lipids and other key nutrients are transported via lymphatics and portal circulation from epithelial cells to other parts of the body.

These phases are included most of the same locations and processes by which all nanomaterials, natural or engineered, have potential to be absorbed regardless of any nutritional value and are also processes that may be exploited in the design and engineering of therapeutics that enter the GIT.

The importance and significance of GIT mucous to the absorption of nanomaterials cannot be overstated [25,26,30]. In mammals including man, orally and rectally administered nano- and microparticles possess surfaces that are either strongly mucoadhesive or non-mucoadhesive: for example, mucoadhesive particles (MAP) administered to mice aggregate in mucus in the center of the GI lumen, far away from the absorptive epithelium, both in healthy mice and in a mouse model of ulcerative colitis (UC) [30]. In contrast, water absorption by the GI tract is rapid and efficient and non-mucoadhesive mucus-penetrating particles (MPP) may be carried and absorbed along with the flow of water (solvent drag) at epithelial surfaces between villi in the small intestine [30]. In the UC mouse model, with high gavage fluid volumes or injection into ligated intestinal loops that are common methods for assessing oral drug and nanoparticle absorption, it was found that both MAP and MPP became well distributed throughout the intestine, indicating that the barrier properties of GIT mucus were compromised [30]. In the mouse colorectum, MPP penetrated the mucus in the deeply in-folded surfaces to evenly coat the entire epithelial surface. Moreover, in a mouse model of UC, MPP were transported preferentially into the disrupted, ulcerated tissue. These results suggest that delivering nanoparticulate or nanoencapsulated drugs in non-mucoadhesive MPP is likely to provide enhanced particle distribution, and thus drug delivery, in the GIT, including to ulcerated tissues, could be enhanced [30].

NATURALLY OCCURRING NANOMATERIALS AND GI ABSORPTION

As noted above, there are quite a number of naturally occurring substances in the environment, in food, and produced by the human body that are in the nanometer (nm) size range [21]. These include nanoparticles from smoke, dusts, food, and water as well as environmental contaminants.

Many globular, dietary proteins are several nanometers in diameter, fatty acids are several nanometers in length, and starch granules have substructures of approximately 30 nm [21]. The double helix structure of DNA has a diameter of 2 nm, and most all viruses have dimensions in the nanometer range [21,22].

Traditional food processing can also generate nanosized structures during emulsification such as colloids, foams, and micelles [30]. Many such materials have a long history of safe consumption: for example, micelles created during the homogenization of milk. Apart from food origins, there are endogenous nanoparticles (NPs) physiologically produced from ions in the mammalian GIT. For example, the precipitation of calcium and phosphate creates nanosized calcium phosphate particles that may be absorbed as such [30]. Thus, the presence and uptake of nutrients with nanoscale dimensions is a normal physiological process.

Food also may contain nanoscale contaminants such as zinc and titanium oxides as well as others and the potential for these contaminants to cause harm is the subject of much debate and current toxicological investigation [25,31–33].

Smoke and dust contain many different types of nanomaterials that may enter the GIT either by ingestion or following inhalation and clearance from the respiratory system [34,35]. Examples of these sources of a wide variety of nanoparticles include diesel exhaust, smoke (including cigarette smoke), dust particles having many different origins and natural aerosols such as sea sprays as well as other forms of bio-liquids.

ENGINEERED NANOMATERIALS AND GI ABSORPTION

GI absorption of nanomaterials is not limited to naturally occurring nanomaterials and over recent years, several different types of engineered nanomaterials have been developed for oral administration to include food, pharmaceutical and medical device applications. These are discussed in more detail in the section on “Engineering Safe Nanoparticles for GI Applications and Therapeutics” below.

There are several challenges in conducting studies on the biological fate and effects of nanomaterials. These challenges include (1) inconsistency in nanomaterials available for study, for example, lot-to-lot variations in important characteristics such as size, shape, and surface properties, and deviations from the labeled description; (2) limitations in quantities available for study; (3) uncertainty as to the proper dose metric (e.g., mass vs. surface area vs. particle concentration); (4) difficulty detecting and quantifying nanomaterials in tissues; and (5) changing chemical and physical properties of nanomaterials with time, handling, and in biological environments [14].

To these challenges, several others are added when studying ingested nanomaterials due to the inherent complexity of the GIT. As ingested nanomaterials transit the GIT, their immediate environment changes in several ways that can affect nanomaterial properties thought to influence biological activity, including size, shape, and surface properties such as charge, catalytic properties, and adsorbed materials (i.e., formation of a lipid and proteinaceous “corona” coating around the nanoparticle) [14]. Examples of

changes in the dynamic environment of the GIT include pH, ionic strength, and composition of GIT fluids, and microflora and digested food matrices with which nanoparticles can interact. Absorptive surfaces also change as nanomaterials pass through the GIT in terms of surface area, absorption mechanisms in play, and thickness of the protective mucin layer [14].

As noted above, in order for ingested nanomaterials, including engineered nanomaterials, to be absorbed from the GIT, they must overcome several barriers. They must survive the low pH, high-ionic environment of the stomach, where they may be dissolved, extensively agglomerated, or coated with macromolecules, thereby altering their surface properties [14]. In the intestine, they must be able to penetrate the mucin layer [36]. Studies have shown that, in general, smaller size favors movement through the mucin layer [37] and neutral particles move the fastest, followed by positively charged particles, with negatively charged particles moving the slowest [38]. Coatings such as chitosan, latex, and polyethylene glycol (PEG) can increase the rate of movement of nanoparticles through mucin [37,39].

Finally, nanoparticles must be able to pass through the epithelial lining of the GIT. Uptake can be passive, through transcellular, paracellular, or cell turnover processes, or active (e.g., clathrin mediated and caveolar mediated). Uptake through the lymphatics can occur in specialized cells in Peyer's patches [14].

Unfortunately, most studies of nanoparticle uptake treat the GIT as a "black box." Nanomaterials are characterized before dosing, and tissues are analyzed over time for the presence of nanomaterials. Because these studies do not follow events within the GIT, no insight is provided as to how the nanoparticles change in the dynamic GIT environment, and most importantly, the characteristics of the particles when they reach the absorptive surfaces of the gut. This greatly limits our ability to gain understanding of how and why nanoparticle characteristics affect their uptake into the body [14].

Among the few studies that have attempted to measure the bioavailability of orally administered nanomaterials, the results have been uniformly low. For example, Schleh et al. [40] measured the bioavailability of native gold particles in sizes ranging from 1.4 to 200 nm, some with surface modifications to produce positive or negative charges. The bioavailabilities of all of the nanoparticles were less than 1%. Findings such as these are difficult to explain because the extent of agglomeration that took place within the GIT, and therefore the actual size of the gold particles when they reached the intestinal epithelium, is unknown [14]. If nearly all of the primary particles were extensively agglomerated into particles too large to penetrate the mucin lining of the GIT or be taken up by any of the particle uptake mechanisms, then the uniformly low bioavailabilities are not surprising.

Roberts et al. [41] reported results from a study in which the oral bioavailability of uncoated and PEG-coated 20 nm gold nanoparticles was compared in mice. The agglomeration state of the gold nanoparticles in each treatment group was followed within the GIT using transmission electron microscopy. Uncoated gold particles agglomerated extensively in the stomach and remained as large agglomerates throughout the GIT. Polyethylene glycol coated (PEGylated) gold nanoparticles remained as primary particles, and in contrast to the large agglomerates in

the uncoated gold-treated animals, were able to penetrate the mucin barrier. Mice treated with PEG-coated gold nanoparticles had significantly higher nanoparticle concentrations in some tissues, but the differences were not striking and the oral bioavailabilities of both the uncoated and PEG-gold were low (<1%). These findings suggest that agglomeration state can influence uptake but that even with well-dispersed particles, bioavailability of nanoparticles is low.

There has been much current investigation as to the circumstances in which the oral uptake of solid nanoparticles is sufficient that they might serve as effective drug delivery vehicles, or for nontherapeutic particles, a significant health risk. This has led to a new generation of studies in which the characteristics (e.g., size, surface charge, and adsorbed materials) of orally administered nanoparticles are examined *in situ*, within the GIT, and critical determinants of uptake are identified [14].

INGESTED NANOPARTICLE TOXICITY

The initial GIT response to the presence of potentially toxic nanoparticles is variable and may exhibit acutely or chronically toxic responses and even be innocuous at the point of entry but target other systemic organs once absorbed.

There is a fundamental gap of knowledge on the health effects caused by the interaction of engineered nanomaterials (ENM) with the GIT. This is partly due to the incomplete knowledge of the complex physical and chemical transformations that ENM undergo in the GIT, and partly to the widespread belief that GIT health effects of ENM are much less relevant than pulmonary effects [42]. However, recent experimental findings, considering the role of the microbiota in gut physiology, shed light on several outcomes of the interaction ENM/GIT [42]. Along with this new information, there is growing direct and indirect evidence that not only ingested ENM, but also inhaled ENM may impact on the GIT [42]. This fact, which may have relevant implications in occupational setting, should be taken into consideration. There are two main aspects to this issue: (1) ENM interaction within the GIT and their possible consequences and (2) relevance of gastro-intestinal effects of inhaled ENMs [42]. Investigation into aspect (1) analyzed how luminal gut-constituents, including mucus, may influence the adherence of ENM to cell surfaces in a size-dependent manner, and how intestinal permeability may be affected by different physico-chemical characteristics of ENM. In this study, cytotoxic, oxidative, genotoxic, and inflammatory effects on different GIT cells, as well as effects on microbiota, were addressed [42]. With respect to issue (2), recent studies show the relevance of gastro-intestinal handling of inhaled ENM and provide evidence of significant excretion in the feces of inhaled ENM and support the hypothesis that GIT should be considered an important target of extrapulmonary effects of inhaled ENM [42]. In spite of recent insights on the relevance of the GIT as a target for toxic effects of nanoparticles, there is still a major gap in knowledge regarding the impact of the direct versus indirect oral exposure. This fact probably applies also to larger particles and dictates careful consideration in workers, who carry the highest risk of exposure to particulate matter [42].

There is limited information on the physical changes of some metallic ENM (Ag, TiO₂, SiO₂ and ZnO) once in contact with the gastro-intestinal fluids [42]. It appears that ion release may occur in the gastric environment, along with size-dependent aggregation and agglomeration. For example, it has been shown that in gastric juice ZnO and Ag undergo dissolution [43–48]. Agglomeration has been shown for TiO₂ and for Ag ENM, dependently from size [43–47,49,50].

Agglomeration has been reported for SiO₂ nanoparticles [51] and de-agglomeration for Ag nanoparticles [43].

TiO₂ ENM have been reported to induce inflammatory changes in the small bowel [43,80] and to enter the systemic circulation to accumulate and cause inflammation and oxidative damage in the liver, kidney, and spleen [52–57]. However, other studies did not detect any adverse effect after oral administration of titanium dioxide, even at very high doses [58–60]. This conflicting data is puzzling and Warheit and Donner [59] observed that negative studies had been performed according to OECD test guidelines, whereas those showing adverse effects were “experimental-type” studies and highlighted the predominant use of mice in studies indicating adverse effects and of rats in those showing no effects, suggesting that differences in susceptibility of exposed animals may contribute to the final result. In addition, commercial test materials were used in studies showing no effects, whereas “home-made” particles were more often used in studies in which adverse effects were observed. However, the presence of substantial adverse effects at doses as low as 1 mg/kg/bw reported by Tassinari et al. [57], and their absence at doses three order of magnitude higher reported by Warheit et al. [59] remain difficult to explain.

ENGINEERING SAFE NANOPARTICLES FOR GI APPLICATIONS AND THERAPEUTICS

Biologics increasingly are being used for the treatment of many diseases. These treatments typically require repeated doses administered by injection. Alternate routes of administration, particularly oral, are considered favorable because of improved convenience and compliance by patients, but physiological barriers such as extreme pH level, enzyme degradation, and poor intestinal epithelium permeability limit absorption. Encapsulating biologics in drug delivery systems, such as polymeric nanoparticles, prevents inactivation and degradation caused by low pH and enzymes of the gastrointestinal tract. However, transport across the intestinal epithelium remains the most critical barrier to overcome for efficient oral delivery. This review focuses on recent advances in polymeric nanoparticles being developed to overcome transport barriers and their potential for translation into clinical use [61].

Nanotechnology is a field of research that has been stressed as a very valuable approach for the prevention and treatment of different human health disorders. This has been stressed as a delivery system for the therapeutic fight against an array of pathophysiological situations. Actually, industry has applied this technology in the search for new oral delivery alternatives obtained upon the modification of the solubility properties of bioactive compounds. Significant works have been made

in the last years for testing the input that nanomaterials and nanoparticles provide for an array of pathophysiological situations. In this frame, this review addresses general questions concerning the extent to which nanoparticles offer alternatives that improve therapeutic value, while avoiding toxicity, by releasing bioactive compounds specifically to target tissues affected by specific chemical and pathophysiological settings. In this regard, to date, the contribution of nanoparticles to protect encapsulated bioactive compounds from degradation as a result of gastrointestinal digestion and cellular metabolism, to enable their release in a controlled manner, enhancing biodistribution of bioactive compounds, and to allow them to target those tissues affected by biological disturbances has been demonstrated [62].

On its way through the gastrointestinal tract, any nanoparticulate drug will encounter a series of barriers before it reaches the capillaries in the subepithelial tissue [63]. The main barriers are the gastric and intestinal milieu, the mucus barrier, the tight junctions blocking paracellular passage, the epithelial cells of the gastrointestinal tract, and finally the subepithelial tissue.

Exposure to nanosized carbon black (CB) particles may increase the risk of cardiovascular diseases by endothelial dysfunction, particularly in susceptible subjects with metabolic syndrome [64]. Vasomotor dysfunction in aorta from obese and lean Zucker rats was investigated after oral exposure to nanosized carbon black (CB). Rats were exposed to 1 or 10 weekly doses of 0, 0.064, 0.64 or 6.4 mg/kg bodyweight and sacrificed 24 hours or 13 weeks later. The exposure to 10 doses of 0.064 or 0.64 mg/kg reduced the acetylcholine-induced vasorelaxation in the lean and obese rats. The half maximal effect concentration values increased by twofold (95% CI: 1.1–3.5-fold) and fourfold (95% CI: 2.3–6.9-fold) in the rats exposed to 0.064 and 0.64 mg/kg compared with the controls, respectively. The rats exposed to 10 doses of 0.64 mg/kg had also 20% (95% CI: 10%–29%) lower maximal effect value compared with the controls. However, the nitroglycerin-induced vasorelaxation and phenylephrine-induced vasoconstriction were not affected in rats exposed to CB. The endothelial dysfunction was not observed in rats sacrificed 13 weeks after the last CB exposure. There was unaltered expression of *Chrm3*, *Nos3*, *Nos2*, *Ccl2*, and *Hmox1* in aorta tissue of CB-exposed rats. In conclusion, repeated oral exposure to CB was associated with endothelial dysfunction in rats, further aggravating the effect of metabolic syndrome [64].

GIT diseases of different types affect the esophagus to the rectum, and the accessory digestive organs such as liver, gall bladder, and pancreas. GI diseases include acute, chronic, recurrent, or functional disorders while covering a broad range of diseases, including the most common acute and chronic inflammatory bowel disease [21]. There are many different options for drug incorporation and drug delivery systems utilizing nanoparticles for oral delivery. Oral drug delivery system is attractive for drug delivery due to its cost effectiveness and relatively easy administration [66]. Oral drug delivery systems are considered complex barrier-exchange systems for taking up the molecules and their absorption in the gut [21]. Variable absorption patterns of the drugs in the GI tract make oral drug delivery challenging due to low solubility, low apparent permeability, and poor bioavailability by indicating the limiting factor for oral chemotherapy [67,68]. To overcome this limitation there is much

interest in nanotechnological-based new and more effective drug carrier systems [65]. Nanotechnology makes it now possible to deliver the hydrophobic drugs, with specific targeting of drugs to particular regions of GIT for transcytosis across the intestinal barriers and intracellular delivery of drugs [69]. Engineered nanoparticles in the size range of 1–100 nm have a broad spectrum of applications in electronics, chemistry, environmental protection, and medicine. In the field of biomedicines, the use of nanoparticles has been growing exponentially and there is some concern for potential adverse effects [70]. To increase the treatment efficacy and to reduce the side effects, nano-based drug delivery systems are being applied and include polymeric, solid lipid, hydrogels, gold, silver nano systems, etc. [65,71]. Metal nanoparticles are currently used in the medical and food industry such as iron, cobalt, copper, zinc, and silica [72]. These types of nanoparticles are physiologically important due to their different synthesis route because of their different physical and chemical properties. Although there are many advantages of the nano-based drug carrier systems but toxicity parameters cannot be overlooked as there are various toxicological routes associated with the exposure of nanoparticles in the human being and environment while toxicological effects on human beings are still ambiguous [65]. Apart from this there are many types of safe nano-based drug delivery systems that are being used as therapeutic agents in the GI diseases. These systems have promising and effectual approach in the field of nano-medicine [65].

Parameters Affecting Nanoparticles in Gastrointestinal Tract

Various parameters such as shape, size, dose, surface characteristics, and translocation are known to play very distinguishing role in the nanoparticles toxicity. These parameters are not still fully understood *in vivo*. Therefore, it is significant to have proper knowledge of nanomaterials interaction with the biological system prior to synthesize. These parameters can influence delivery efficiency and distribution of drugs. In the designing of nanoparticles a few key points should be considered such as appropriate surface charge and that specific ligands be in the circulatory system. Nanoparticles can escape from the clearance mechanism and opsonisation, resistant to drugs, and other related parameters. Therapeutic applications of nanoparticles are dependent on these parameters when interacting with the biological system [65].

The increasing interest in nanoparticles for advanced technologies, consumer products, and biomedical applications has led to great excitement about potential benefits but also concern over the potential for adverse human health effects [73]. The gastrointestinal tract represents a likely route of entry for many nanomaterials, both directly through intentional ingestion or indirectly via nanoparticle dissolution from food containers or by secondary ingestion of inhaled particles [73]. The gastrointestinal tract is a site of complex, symbiotic interactions between host cells and the resident microbiome and increased utilization of nanoparticles may lead to increased environmental contamination and unintentional ingestion via water, food animals, or fish [73]. Toxicological evaluation of nanoparticles must take into consideration not only absorption and systemic organ accumulation but also the potential for alteration of gut microbes and the effects of this perturbation on the host [73]. The existing

literature was evaluated for evidence of toxicity based on these considerations. Focus was placed on three categories of nanomaterials: nano-metals and metal oxides, carbon-based nanoparticles, and polymer/dendrimers, with emphasis on those particles of greatest relevance to gastrointestinal exposures [73].

Intestinal mucus, as introduced above, is a complex, natural network of highly branched glycoproteins, lipids, cellular, and serum macromolecules, and is the first barrier through which ingested nanoparticles must pass [74]. Surface charge can play a crucial role [75]. Net neutral or positive surface charge prevents mucoadhesion, favoring penetration, whereas passage of negatively charged hydrophilic and lipophilic compounds is hindered. Small nanoparticles also penetrate more easily than large ones [75]. Mucin interaction with adhesive nanoparticles and larger particulates can disrupt the “bottle-brush” architecture of mucus, possibly enabling penetration upon subsequent exposures [76,77]. This is dependent upon particle type. Jachak et al. [78] found that metal oxide NPs and two types of single-walled carbon nanotubes (SWCNTs) were trapped in human mucus by adhesive interactions, not steric obstruction. In contrast, ZnO nanoparticles rapidly penetrated airway mucus layers, which may account for ZnO’s general toxicity.

Whether in the GIT or in a cultured microenvironment, most all nanoparticles will develop a “corona” of adsorbed proteins as well as other small molecules, and ions [79–82]. This association can sequester nutrients, etc., complicating interpretation of cell culture results [83] and create an “epitope map,” or complex biologically active entity that influences the *in vivo* response [82,84]. The effects of the protein corona are variable on biological systems. In some cases, cytotoxicity is reduced, perhaps by decreasing cellular nanoparticle uptake [85–88] or by mitigating cell membrane damage [81]. Lundqvist et al. [81] found that the protein corona on 50 nm carboxyl-modified polystyrene nanoparticles varied in relation to particle size and surface modification. Highly abundant plasma proteins such as inter-alpha-trypsin inhibitors, serum albumin, clusterin, and vitronectin appear to be common to all coronas, while less abundant proteins appear to vary with nanoparticle size and surface characteristics [81]. In one study of polystyrene nanoparticles in human plasma, Zhang et al. [89] classified nanoparticles with respect to protein coating based on size and surface properties of the parent particles. In addition, the protein corona was demonstrated to be at equilibrium within 5 minutes of nanoparticle exposure. Variable coronal protein composition may lead to some of the different biological effects of nanoparticles that otherwise seem identical [90]. These studies further emphasise the need for adequate characterisation of nanoparticles both before and after exposure to culture media or biological fluids [81].

Animals and human studies have shown that after inhalation and through oral exposure, nanoparticles are distributed to the liver, heart, spleen, and brain in addition to lungs and gastrointestinal tract [92–94]. Often, during clearance of nanoparticles from the body, the components of the immune system are activated. During metabolism, some nanoparticles tend to congregate in the liver tissues [92–96].

Copper oxide nanoparticles are used in semiconductors, antimicrobial reagents, heat transfer fluids, and intrauterine contraceptive devices [97]. Experimentally, copper nanomaterials have been documented to possess toxic effects on the liver and

kidney [98]. Nano-copper has resulted severe impairment in liver, kidney, and spleen in experimental animals. After oral administration and interacting with gastric juice, highly reactive ionic copper is formed, which is then accumulated in the kidney of exposed animals [99,100]. In one *in vitro* study, copper oxide nanoparticles (50 nm) have been reported as being genotoxic and cytotoxic along with disturbing cell membrane integrity and inducing oxidative stress [91,101].

There is considerable research interest in the area of oral drug delivery using a variety of nanoparticle delivery systems as carriers. Oral nanoparticles have been used as a physical approach to improve the solubility and the stability of active pharmaceutical ingredients (APIs) in the gastrointestinal juices, to enhance the intestinal permeability of drugs, to sustain and to control the release of encapsulated APIs allowing the dosing frequency to be reduced, and finally, to achieve both local and systemic drug targeting. Numerous materials have been used in the formulation of oral nanoparticles leading to different nanoparticulate platforms based on the formulation and the characterization of polymeric, lipid, and inorganic nanoparticles to facilitate oral delivery of pharmaceuticals and therapeutic genes [102,103].

The mucosal route is gaining attention for noninvasive drug delivery via the oral, nasal, pulmonary or vaginal routes [104,105]. At the same time, nanoparticle technology has also come to the forefront as a viable drug delivery strategy, presenting opportunities for controlled release, protection of active components from enzymatic or environmental degradation and localized retention. Nanoparticle fabrication methods are readily scalable and applicable to a broad range of drugs. Of all the nanoparticle drug delivery approaches, polymeric nanoparticles have gained significant importance as they are biodegradable, biocompatible, and because formulation methods are more widely available; the range of applications has been expanding to include a variety of chemical drug classes and dosage forms [106]. Chitosan-based NP are particularly appropriate for the mucosal route, with their low toxicity, mucoadhesion, and tunable physical properties. There are many examples of chitosan-based nanoparticles developed for use in the treatment of cancer, gastrointestinal diseases, pulmonary diseases, drug delivery to the brain, and ocular infections. Recent research on chitosan-based nanoparticles for nonparenteral drug delivery is based on an expanding understanding of chitosan properties and methods of chemical or physical modification, which are applied to optimize nanoparticle loading of pharmaceuticals and to enhance their release features [104,105].

Formulation into nanoparticles can improve drug stability in the harsh GIT environment, providing opportunities for targeting specific anatomical sites, increasing drug solubility and bioavailability, and providing sustained release of pharmaceuticals [103]. However, the unique and diverse physiology throughout the GI tract, including wide variation in pH, mucus that varies in thickness and structure, numerous cell types, and various physiological functions are both a barrier to effective delivery and an opportunity for nanoparticle design.

Oral delivery is preferred for local administration of intestinal therapeutics [107]. Lipid nanoparticles, which have been previously shown to deliver siRNA to intestinal epithelial cells, have potential to treat intestinal disease. It is unknown, however, whether the oral delivery of these particles is possible. To better understand the fate of

lipid nanoparticles in the GIT, Ball et al. [107] studied delivery under deconstructed stomach and intestinal conditions *in vitro*. Lipid nanoparticles remained potent and stable following exposure to solutions with pH values as low as 1.2. Efficacy decreased following exposure to “fed,” but not “fasting” concentrations of pepsin and bile salts. The presence of mucin on Caco-2 cells also reduced potency, although this effect was mitigated slightly by increasing the percentage of PEG in the lipid nanoparticle. Mouse biodistribution studies indicated that siRNA-loaded nanoparticles were retained in the GI tract for at least 8 hours. Although gene silencing was not initially observed following oral LNP delivery, confocal microscopy confirmed that nanoparticles entered the epithelial cells of the mouse small intestine and colon. Together, these data suggest that orally delivered LNPs should be protected in the stomach and upper intestine to promote siRNA delivery to intestinal epithelial cells [107].

The viscoelastic mucus secretions coating exposed organs such as the lung airways and the female reproductive tract can trap and quickly eliminate not only foreign pathogens and ultrafine particles but also particle-based drug delivery systems, thus limiting sustained and targeted drug delivery at mucosal surfaces. To improve particle distribution across the mucosa and enhance delivery to the underlying epithelium, many investigators have sought to develop nanoparticles capable of readily traversing mucus. The first synthetic nanoparticles shown capable of rapidly penetrating physiological mucus secretions utilized a dense coating of PEG covalently grafted onto the surface of preformed polymeric nanoparticles. In the decade since, PEG has become the gold standard in engineering mucus-penetrating drug carriers for sustained and targeted drug delivery to the lungs, gastrointestinal tract, eyes, and female reproductive tract [108]. Huckaby and Lai [108] have recently summarized the history of the development of various PEG-based mucus-penetrating particles and have highlighted the key physico-chemical properties of PEG coatings and PEGylation strategies to achieve muco-inert PEG coatings on nanoparticle drug carriers for improved pharmaceutical and gene delivery at mucosal surfaces.

Different materials have been entrapped into lipid nanoparticles, extending from lipophilic and hydrophilic molecules, including labile compounds, such as proteins and peptides [109,110]. Solid lipid nanoparticles (SLNs) were developed at the beginning of the 1990s as an alternative carrier system to liposomes, emulsions, and polymeric nanoparticles as a colloidal system for controlled drug delivery. SLNs consist of a solid lipid, where the drug is normally incorporated, with an average diameter below 1 μm [111,112]. Per oral administration forms of SLN may include aqueous dispersions or SLN-loaded traditional dosage forms, e.g., tablets, pellets, or capsules [113].

Nanostructured lipid carriers (NLCs) are drug delivery systems composed of both solid and liquid lipids as a core matrix. NLCs disclose some advantages for drug therapy over conventional carriers, including increased solubility, the functional groups lead to pH-dependent activities that are useful for targeting different parts of the gastrointestinal tract [114].

Chitosan is a polymer that is very commonly used in oral nanoparticles. Mechanism of chitosan nanoparticles transport across the gastrointestinal tract is most probably through adsorptive endocytosis. Electrostatic interaction between positively charged chitosan and negatively charged sialic acid of mucin causes

association of chitosan nanoparticles to the mucus layer and then internalization via endocytosis [115,116]. Behrens et al. indicated that chitosan nanoparticles functional groups lead to pH-dependent activities that are useful for targeting different parts of the gastrointestinal tract [117].

SUMMARY AND CONCLUSIONS

In summary, the recent literature is beginning to include some significant advances in the knowledge of GIT exposure to nanomaterials. Substantial gaps remain to be investigated and elucidated, however. *In vitro* mechanistic studies and oral studies primarily in rodents have been principal areas of advancement leaving much more to be done. Nevertheless, current research demonstrates that:

- Biopersistence (long-term retention of nanoparticles in target tissues) is a critical factor in determining whether nanoparticles penetrate cells and are retained long enough to provide the continuing inflammatory signals and other biological stimuli which may lead to pathological changes.
- Nanoparticles can penetrate the GIT walls and may both produce therapeutic effects or induce adverse events such as cellular oxidative stress and the initiation of apoptosis or cell necrosis.
- Modification of surface activity of otherwise hydrophobic nanoparticles (e.g., formation of a protein “corona”) can modify the toxicity potential of nanoparticles.
- There currently is no clear pattern of structure activity relationships (SAR) which would permit predictability of nanoparticles toxicity.
- There is a lack of useful chronic oral exposure studies with nanomaterials, especially those which clearly demonstrate dose-response toxicological effects and provide the determination of well-characterized no adverse effect levels (NOAELs).
- Adequate characterization of nanomaterials administered in either *in vitro* or *in vivo* experimental studies remains critical to the interpretation of the outcomes and comparison of findings.

As our understanding of the effects of nanomaterials on normal GIT function improves so will our ability to recognize and potentially treat or correct abnormal intestinal function. Advances have been made in the selective targeting of pharmaceuticals using a variety of nanoparticles. Toxicological issues are being investigated. GIT-engineered nanotechnologies are certain to have major impacts toward advancing both our knowledge of human intestinal function and the quality of life of those with conditions where intestinal function is abnormal in some manner.

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